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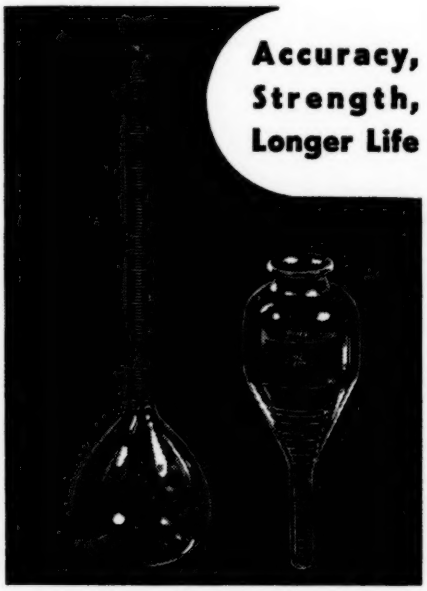

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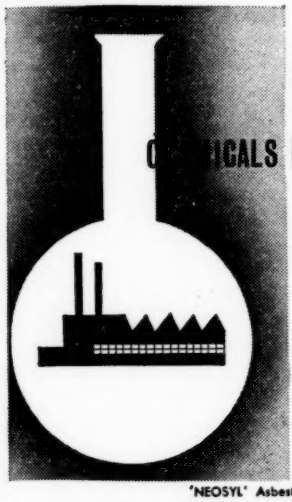

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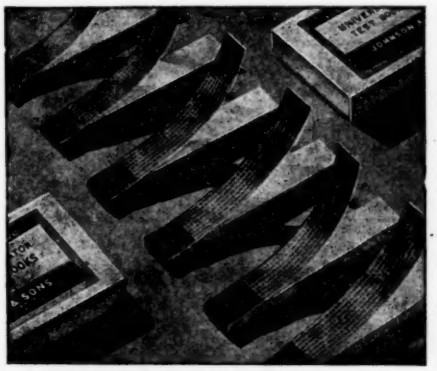

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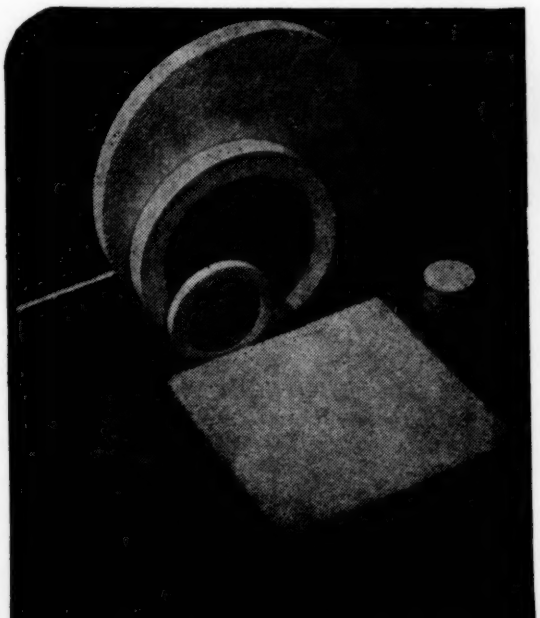

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297. *The Adsorption of Acetic Acid and* n-*Butylamine from Aqueous Solutions by Activated Charcoals.*

By J. J. Kipling.

Adsorption by charcoal of acetic acid and *n*-butylamine from aqueous solutions has been investigated over a range of pH and other conditions. Adsorption takes place only through the undissociated molecules. It is concluded that the variation of degree of adsorption with pH is a function of the electrochemical properties of the solution only and does not depend on changes in the nature of the charcoal surface. On this basis, equilibrium adsorption under any stated conditions of pH and concentration can be calculated from the adsorption isotherm.

It is also shown that (within the practical limits of the activation process) the adsorption capacity of the charcoal for these substances increases with decreasing activation yield, but is not affected by the temperature of activation.

Adsorption by charcoal from solutions may be of different types according to the nature of both solute and solvent. The present study is restricted to one type only, *viz.*, that of weak organic electrolytes in aqueous solution. It is concerned with ascertaining, first, whether both ions and undissociated molecules are adsorbed, and secondly, whether the adsorbate is held by " physical " or " chemical " forces (using Brunauer's definitions, " The Adsorption of Gases and Vapours ", Oxford, 1944) or by electrochemical forces. Adsorbates of small molecular size were chosen for this work, so that complications due to steric effects might be avoided.

Fromageot and Wurmser (*Compt. rend.*, 1924, **179**, 972) investigated the adsorption of oxalic acid on charcoal, but offered no adequate explanation of their results, which, however, are readily interpreted in the light of the present work.

Phelps and Peters (*Proc. Roy. Soc.*, 1929, **124**, *A*, 554) found that the adsorption of the lower fatty acids and of simple aliphatic amines depends on the pH of the solution and closely follows the ionic dissociation curves of the acids and bases respectively. They concluded, therefore, that adsorption takes place through the undissociated molecule only. If this conclusion is correct, it should be possible to deduce the extent of adsorption at any pH from the adsorption isotherm of the free acid or base. In referring to the fall in adsorption of the acids with increasing pH, Phelps and Peters observe that " the fall in adsorption may be correlated almost quantitatively with the percentage of un-ionised molecules present." This seems to involve a tacit assumption that the extent of adsorption is proportional to the number of undissociated molecules present, whatever their concentration in the solution may be, which is equivalent to assuming that the adsorption isotherms of the acids are linear. We shall consider whether their main conclusion remains applicable if the observed isotherm is used as a basis for calculation.

The work of Phelps and Peters implies that the adsorption from these solutions is probably similar to that of gases like carbon tetrachloride. Such gases are held by " physical " forces and their adsorption does not depend on the precise chemical nature of the charcoal surface; the most important factor has been shown to be the activation yield (paper in preparation). On the other hand, King (*J.*, 1937, 1489) finds that adsorption of acetic acid on charcoal activated by oxygen depends on the temperature of activation, and that increase in adsorption of acetic acid is accompanied by decrease in adsorption of ammonia. He concludes that the chemical or electrochemical nature of the charcoal surface may influence adsorption.

To investigate these two problems, experiments have been designed to show the effect of the following variables on the adsorption of a simple acid and a simple amine: (*a*) pH of solution, (*b*) activation conditions, (*c*) activation yield, (*d*) nature of raw material from which the charcoal was made, and (*e*) concentration of solution.

Experimental.

Reagents.—The adsorbates were acetic acid and *n*-butylamine, which were chosen as having approximately equal electrolytic strength and molecular size. The solutions were buffered with sodium acetate and hydrochloric acid respectively.

The charcoals were used in granular form, graded 8—18 B.S.S. They were prepared from carbonised nutshell and briquetted coal by steam activation at 890° in the laboratory, as described elsewhere. Special charcoals were activated at 700° and 1000°. To extend this range, a further charcoal which had been activated at 890° was heated with steam at 600° for 3 hours to allow it to assume the surface structure appropriate to that temperature; activation is too slow at 600° for the whole of the activation to be carried out at that temperature. A few results were also obtained with commercially activated charcoals.

Methods.—In general, the charcoal and the buffered solution were shaken together for 12 hours. The (equilibrium) pH of the solution was then measured. For acetic acid, the adsorption was determined from the difference in total acetate concentration before and after adsorption. The method described by

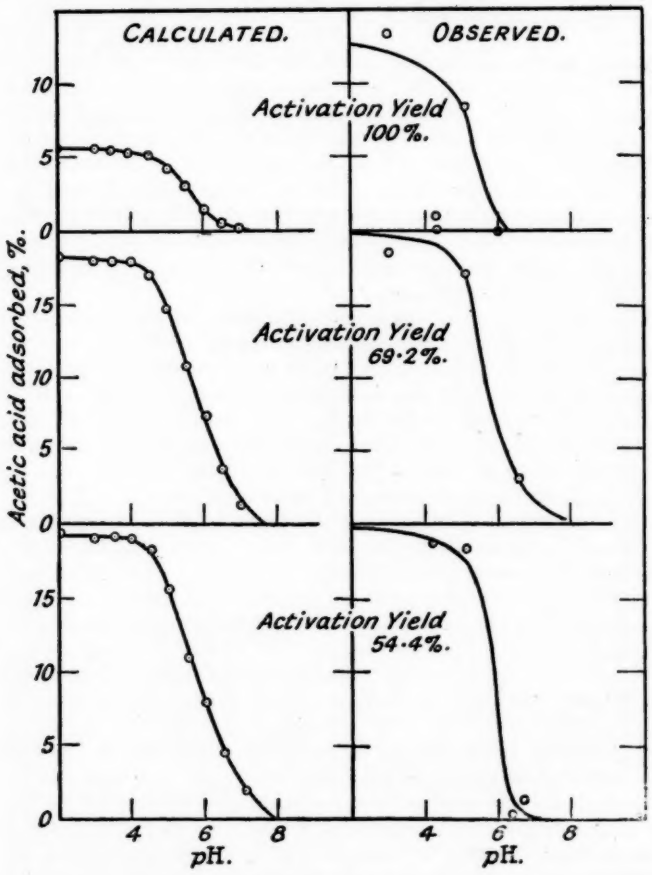
Phelps and Peters (*loc. cit.*) was followed, except that the titrations were carried out electrometrically. A similar method was used for determining " total butylamine " (*i.e.*, free base + butylamine hydrochloride). This required a determination of the dissociation constant of *n*-butylamine; an approximate value of $4{\cdot}2 \times 10^{-4}$ was obtained.

Results.—The results below have been calculated to test the assumption that only undissociated acid or base is adsorbed by the charcoal. Unless otherwise stated, concentrations refer to " total acetate " or " total butylamine ".

Effect of pH. For acetic acid, adsorption is at a maximum in acid solutions, but falls rapidly between pH 5 and 6, and is negligible in alkaline solutions. This is shown in Fig. 1 and Table I. For butylamine, there is a continuous fall in adsorption from pH 12 to zero at about pH 6 (Fig. 2, Table II).

FIG. 1.

Effect of pH *on adsorption of acetic acid.*



The curves of adsorption against pH will be described in terms of two quantities: (*a*) the value of the maximum adsorption; (*b*) the pH of the "point of inflexion " of the curve, taken arbitrarily as the point at which adsorption is one-half of the maximum value.

Effect of activation yield. The point of inflexion is independent of the activation yield. The maximum adsorption increases with continued activation. These results apply to both acetic acid and butylamine (Fig. 3, Tables I and II).

Effect of concentration of solution. Most of the work was done with N-solutions. For acetic acid, however, the work on nutshell charcoals was repeated with N/10-solutions. The same results were obtained, except that the maximum adsorption was found to be less in this case. This depends on the isotherm for adsorption of unbuffered acetic acid. Representative isotherms are shown in Fig. 4. Similar isotherms were obtained for *n*-butylamine.

Effect of temperature of activation. There is no appreciable change in point of inflexion or total adsorption which can be attributed to the temperature of activation within the range practicable for steam activation, *viz.*, 600—1000° (Table III).

Nature of the activation process. The curve for adsorption of acetic acid against pH for a " chemically activated " charcoal ("Sorbonorit") is precisely similar in form to those for the other charcoals investigated, having a maximum adsorption of 15·7% and a point of inflexion at pH 6·0.

FIG. 2.

Effect of pH *on adsorption of* n*-butylamine.*

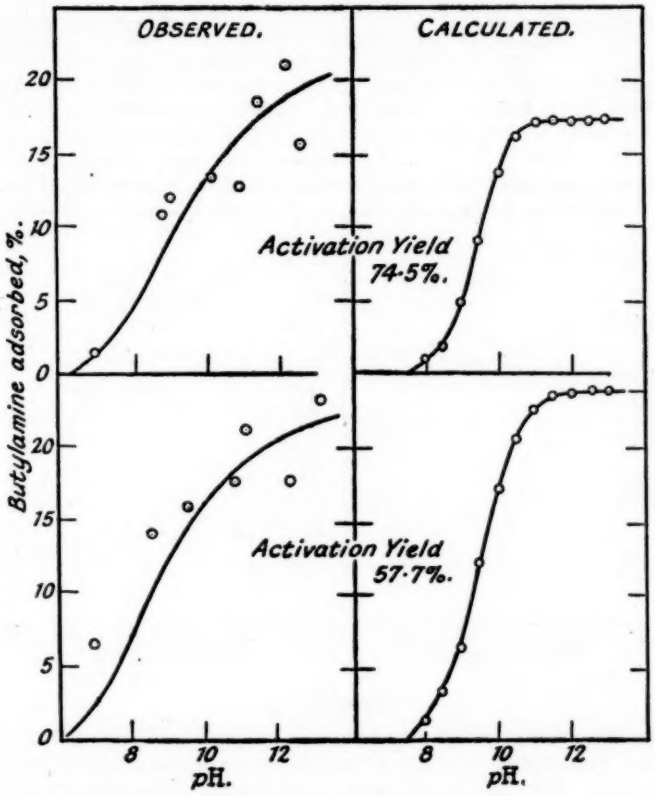


TABLE I.

Adsorption of acetic acid on charcoals.

Activation yield (%).	Maximum adsorption (%).	pH at point of infln. Obs.	pH at point of infln. Calc.	Activation yield (%).	Maximum adsorption (%).	pH at point of infln. Obs.	pH at point of infln. Calc.
On nut charcoals from N-solution.							
100	12·5	5·3	5·6	54·4	19·5	6·0	5·8
81·9	18·7	5·9	—	43·2	21·2	5·8	—
69·2	19·5	5·9	5·8	24·4	25·0	6·0	—
On nut charcoals from N/10-solution.							
100	2·6	5·5	4·9	54·4	8·7	5·4	5·4
81·9	7·0	5·8	—	38·8	8·0	6·1	—
69·2	9·2	5·7	5·3	22·0	8·8	5·8	—
On coal charcoals from N-solution.							
100	7·0	5·6	5·6	47·2	20·0	5·6	5·7
75·2	13·5	5·6	6·1	37·6	19·2	5·6	—
57·2	14·8	6·0	—	26·8	17·5	5·9	—

TABLE II.

Adsorption of n*-butylamine on nut charcoals from* N*-solution.*

Activation yield (%).	Maximum adsorption (%).	pH at point of infln. Obs.	pH at point of infln. Calc.	Activation yield (%).	Maximum adsorption (%).	pH at point of infln. Obs.	pH at point of infln. Calc.
100	4·3	10·5	10·0	57·7	24·8	8·8	9·5
74·5	20·6	9·3	9·4	37·9	28·5	8·5	—
65·6	22·5	8·7	—	23·4	31·5	8·8	—

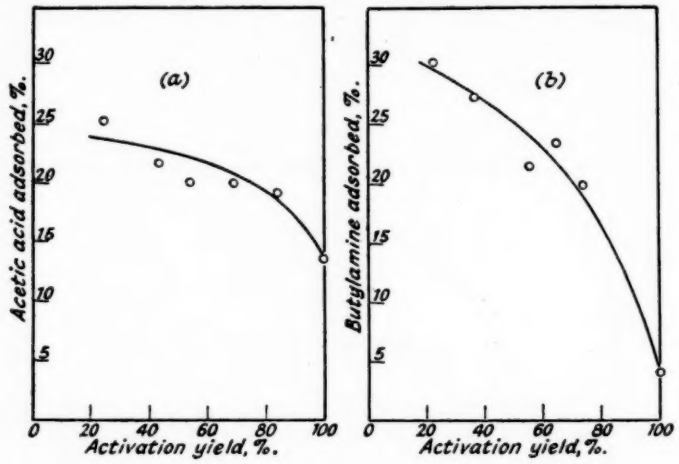
TABLE III.

Effect of temperature of activation on adsorption of acetic acid on nut charcoals from N-*solution.*

Temp. of activation.	Activation yield (%).	Maximum adsorption (%).	pH of point of infln.	Temp. of activation.	Activation yield (%).	Maximum adsorption (%).	pH of point of infln.
1000°	48·9	17·7	5·9	700°	55·2	17·0	5·9
890	43·2	19·5	5·9	600	59·0	18·5	5·8

FIG. 3.

Effect of activation yield on adsorption of acetic acid and n-*butylamine.*



DISCUSSION.

In Figure 1 the observed variation of adsorption of acetic acid with pH is compared with the calculated results. The calculations are based on the experimentally determined isotherm of unbuffered acetic acid. The two curves agree very closely. The calculated adsorption does in fact also follow the dissociation curve of the acid very closely. This is due partly to the small pH range through which the fall in free acetic acid concentration takes place and partly to the shape of the isotherm, most of which is nearly linear over a wide range of concentration. Thus the approximation of Phelps and Peters does hold very closely for this particular case, but it is important to realise that it might not hold for substances with isotherms of a very different shape or with shallower dissociation curves.

The corresponding curves for butylamine are shown in Fig. 2. The observed curves follow the calculated curves qualitatively, but drop less sharply than expected from maximum to zero adsorption. Thus the general conclusion reached for acetic acid, that adsorption takes place through the undissociated molecule, is confirmed. The slight quantitative discrepancy is probably due to the basic character of the mineral content of the charcoals. This will raise the pH of the solution which first reaches the charcoal surface and so give an abnormally high adsorption. When an equilibrium pH is established, some desorption of butylamine should take place. It is shown in the following paper, however, that such desorption is slow and hence a false equilibrium is maintained.

The experimental data considered up to this point are thus in complete accord with the theory that only the undissociated acid or base is adsorbed and that the extent of adsorption follows an isotherm of the Langmuir type. This theory will account for the observation of " hydrolytic adsorption " in the case of salts of a weak acid or base. This phenomenon is essentially the adsorption of some component from the solution of a salt which results in a change in the pH of the solution (see, *e.g.*, Bartell and Miller, *J. Amer. Chem. Soc.*, 1922, **44**, 1866). It has been connected with specific chemical structures of the charcoal surface (*idem*, *J. Physical Chem.*, 1924, **28**, 992). Although such an explanation may be required for some types of adsorption (*e.g.*, of strong acids and bases), it is clearly unnecessary for salts of weak acids and bases.

There is quantitative evidence that the charcoal surface exhibits no specific acidic or basic

behaviour. Fig. 5 shows that equal numbers of molecules of acetic acid and butylamine are adsorbed on unit weight of a given charcoal. It is probable (from the shape of the isotherms) that these substances form only a unimolecular layer on the charcoal surface, and it is likely that they then occupy the same area per molecule as in the corresponding layers on liquid surfaces. This is 20·5 sq. A./molecule for both substances (Adam, " The Physics and Chemistry of Surfaces ", Oxford, 1941). Hence they can separately occupy equal areas of charcoal surface. We conclude, therefore, that the adsorption of acetic acid and butylamine on charcoal is independent of the chemical and electrochemical nature of the surface and that possibly the surfaces of these charcoals are all, in this respect, essentially similar.

FIG. 4.

Adsorption isotherms of acetic acid on charcoal.

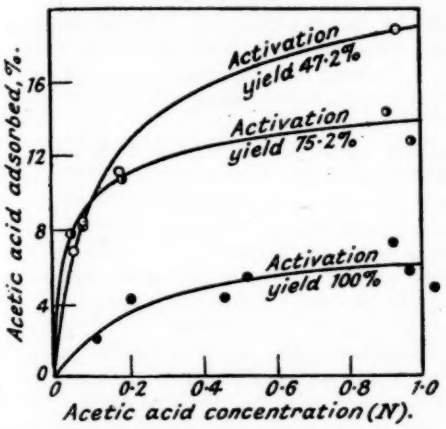
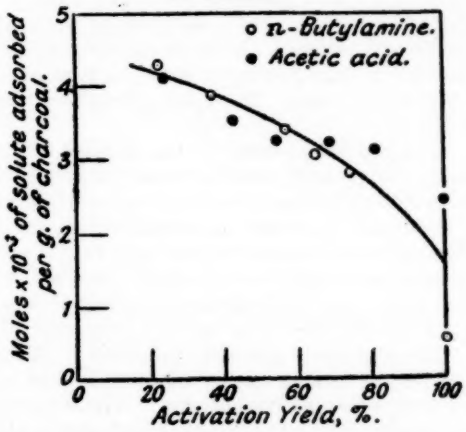


FIG. 5.

Molar adsorption of acetic acid and n-butylamine on charcoal.



This conclusion is supported by two further results. (i) The same phenomena are observed when the charcoal is made from different raw materials or by different methods of activation. (ii) The easy reversibility of adsorption referred to in the following paper confirms that this adsorption is of a physical and not a chemical nature.

Our conclusions do not accord with those of King. We would tentatively suggest that his varying adsorption according to the temperature of activation may be due to the different degrees of activation obtained as a result of variation in the rates of oxidation as the temperature varied.

Finally, it should be emphasised that this discussion applies to the adsorption of acetic acid and butylamine and does not necessarily apply to the adsorption of all solutes.

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PHYSICAL CHEMISTRY LABORATORY, OXFORD. (*Received, June 16th,* 1947.]

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298. *The Behaviour of Charcoal Columns in the Adsorption and Desorption of Acetic Acid in Aqueous Solution.*

By J. J. KIPLING.

Studies of the adsorption on charcoal columns of acetic acid from a stream of aqueous solution and of its subsequent desorption into pure water are directly relevant to a number of practical problems.

The initial penetration (" breakdown ") phenomena of the column can be interpreted in terms of a general theory of adsorption (based on the assumption that the rate of diffusion of the adsorbate to the adsorbing surface is fast relative to the rate of flow of the carrier stream). Theoretical predictions of the effect on the breakdown time of a column of (*a*) column length, (*b*) flow-rate of the stream, (*c*) initial concentration of the stream, and (*d*) granule size of the charcoal are compared with experimental results.

The distribution of the adsorbate on the column depends on the precise shape of the adsorption isotherm. The distribution can be calculated from the experimentally determined isotherm if it is assumed that equilibrium between the stream and the charcoal is attained

rapidly relative to the rate of flow of the stream. This theoretical treatment gives results in good accord with those determined experimentally. It can be applied to distribution during adsorption and during desorption by a stream of pure solvent, both for uniform and for non-uniform initial distribution of adsorbate.

THE preceding paper confirmed the view that the adsorption on charcoal of acetic acid from aqueous solution takes place through the undissociated molecule only. This work referred to adsorption under conditions of strict equilibrium, whereas the present paper considers the phenomena arising from the adsorption of acetic acid from solutions flowing through packed columns of granular charcoal, *viz.*, " breakdown " phenomena, and the distribution of adsorbate during both adsorption and desorption. This work has a direct bearing on numerous practical problems of recovery and purification of both solutes and solvents.

The corresponding case for gaseous adsorbates has been described in two papers (Danby, Davoud, Everett, Hinshelwood, and Lodge, *J.*, 1946, 918; Barrow, Danby, Davoud, Hinshelwood, and Staveley: *J.*, 1947, 401), which form the basis of this comparison. The mathematical treatments presented in these papers were developed with reference to the general problem of adsorption from flowing streams, with no assumptions limiting their application particularly to the gaseous phase. In outline, therefore, they form the theoretical background of the present work.

The general problem of the passage of an adsorbate carried in an inert stream through a column of granular adsorbent can be given a strict mathematical treatment, but this leads to a differential equation which is, in general, intractable. By the use of approximations, however, it is possible to derive equations which can be compared directly with experimental results. These approximations depend upon one or other of the following assumptions: either (*a*) that, for substances which are strongly adsorbed, adsorption can be treated as an irreversible process of definite rate, or (*b*) that in any adsorption or desorption process, equilibrium is established at a rate fast compared with the rate of flow of the adsorbate through the column.

The first assumption is applicable to the initial breakdown phenomena of reacting columns (*i.e.*, to phenomena connected with the first appearance of detectable traces of the adsorbate in the stream leaving the column). The second assumption relates to the distribution of reactant or adsorbate along the column as a function of time, both in adsorption and desorption. In both cases the shape of the adsorption isotherm is of fundamental importance. We shall consider these in turn in relation to the adsorption of acetic acid on charcoal from aqueous solution.

Breakdown Phenomena.

Assumption (*a*) above is applicable to the treatment of the initial breakdown of columns. It applies when the shape of the adsorption isotherm is such that the total capacity of the charcoal for the adsorbate is very large. Adsorption then takes place very readily, while desorption, under normal conditions of flow, is very slow, so that the former can be regarded, to a first approximation, as an irreversible process.

The successful application of this approximate treatment to the adsorption of acetic acid depends on: (i) the shape of its adsorption isotherm, (ii) its rate of adsorption. The shape of the adsorption isotherm of acetic acid on charcoal justifies the use of the approximation that adsorption is effectively an irreversible process *under these conditions* (*i.e.*, a steady flow of solution through the column), provided that the solutions used be not very weak.

A preliminary investigation of the rate of adsorption showed it to be fast relative to the rates of percolation used. Thus, for charcoals of the kind used in these experiments, but in the coarser grading of 8—18 mesh B.S.S., 80% of the total adsorption took place within 15 minutes. The rate appeared to be faster for more active charcoals, and a faster rate would be expected if finer grading were used.

The mathematical treatment of the general problem has been given in full by Danby, Davoud, Everett, Hinshelwood, and Lodge (*loc. cit.*). The basic equation relating the breakdown time, τ, of a column of length λ is:

$$\tau = \frac{1}{kc_0} \cdot [\ln (e^{kN_0\lambda/L} - 1) - \ln (c_0/c' - 1)]$$

where c_0 is the concentration of adsorbate entering the column,
c' is the concentration of adsorbate leaving the column at breakdown,
L is the linear flow rate of the solution,
N_0 is the number of active centres of unit activity per c.c. of material,
and k is a constant, except under certain conditions, specified below.

If, as proves to be the case in practice, $e^{kN_0\lambda/L}$ is much greater than 1, we can re-write this equation as

$$\tau = \frac{1}{kc_0}.\left[\frac{kN_0\lambda}{L} - \ln\,(c_0/c' - 1)\right] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

From this equation relations can be derived for the effect of each variable on the behaviour of the column.

Effect of column length. A linear relation is deduced :

$$\tau + \tau_0 = N_0\lambda/c_0L \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where $$\tau_0 = [\ln\,(c_0/c' - 1)]/kc_0$$

Effect of flow rate. The fundamental property of charcoal columns, rather than the breakdown time, is the breakdown volume, Y. This is the volume of solution which passes through the column before breakdown is reached. It is related to the breakdown time by the equation

$$Y = F\tau$$

where F is the total flow rate of the solution through the column and is related to the linear flow-rate by the equation

$$F = AL$$

where A is the cross-sectional area of the column.

Equation (1) can now be re-written in the form

$$F\,.\,\ln\,(c_0/c' - 1) = -kc_0Y + kAN_0\lambda \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Rates of diffusion are slower in solution than in the vapour phase and it is therefore necessary to use the modified rate constant for adsorption, k_D, in equation (3), where :

$$k = k_DL^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

This gives

$$F\,.\,\ln\,(c_0/c' - 1) = -k_DL^{\frac{1}{2}}c_0Y + k_DL^{\frac{1}{2}}AN_0\lambda$$

Hence

$$Y = \frac{AN_0\lambda}{c_0} - \frac{F^{\frac{1}{2}}A^{\frac{1}{2}}\,.\,\ln\,(c_0/c' - 1)}{k_Dc_0}$$

which can be re-written in the form :

$$Y = Y_0 - KF^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

where Y_0 and K are constants.

Effect of initial concentration of solution. The effect of variation of breakdown time with initial concentration cannot be expressed in any simpler form than that of equation (1). From this we see that a plot of τ against $1/c_0$ should give a straight line when c_0 is small [*i.e.*, when $\ln\,(c_0/c' - 1)$ is much less than $kN_0\lambda/L$], falling off towards the $1/c_0$ axis as c_0 increases, *i.e.*, towards the origin.

Effect of granule size. Reduction of granule diameter increases the accessibility of the adsorption sites, although the number of active centres per c.c. remains unchanged. Accessibility might be expressed approximately as the ratio

$$\frac{\text{external surface of granule}}{\text{mass of granule}} = \frac{\pi d^2}{1/6\pi d^3} = \frac{6}{d}.$$

Hence we can write k^*/d for k in equation (1), which becomes

$$\tau = \frac{N_0\lambda}{c_0L} - \frac{d\,.\,\ln\,(c_0/c' - 1)}{k^*c_0}$$

which is of the form

$$\tau = \tau_0' - k_1d \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

where τ_0' and k_1 are constants.

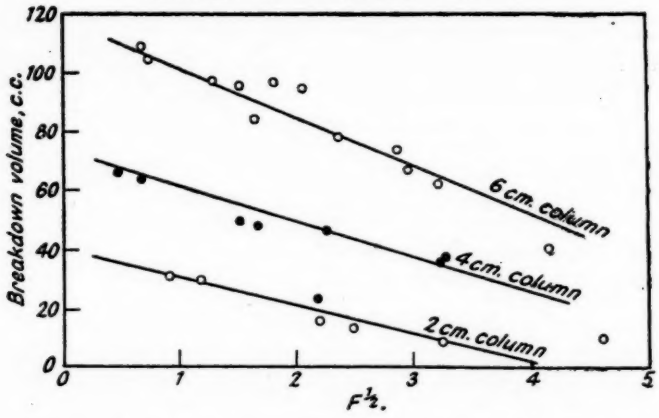
Note. The equations used throughout refer to a breakdown criterion based on the concentration of solute in the effluent. The experimental breakdown criterion depends on the total amount of solute penetrating the column. Slightly more complicated equations apply to this case, but differ from those given only in the value of some of the constants. The difference is small and does not affect the form of the equations.

Experimental.—The experiments were carried out with columns of steam-activated nutshell charcoal and unbuffered solutions of acetic acid. The columns were supported on a sintered-glass plate in a tube 16 mm. in diameter. The charcoals were carefully graded before use and then well washed with distilled water to remove fine particles. The columns were formed from the wet charcoal, which was packed to maximum density. Solutions from a reservoir were allowed to percolate through the columns at controlled rates. The criterion of breakdown was neutralisation by the effluent stream of 1 c.c. of N/100-sodium hydroxide, with phenolphthalein as indicator. The final volume was measured after breakdown.

The following conditions applied throughout except where otherwise stated: Grading of charcoal: 40—80 mesh B.S.S. Flow-rate of solution: 2 c.c./min. Column length: 4 cm. Concentration of acetic acid: N/10.

FIG. 1.

Effect of flow rate on breakdown volume.

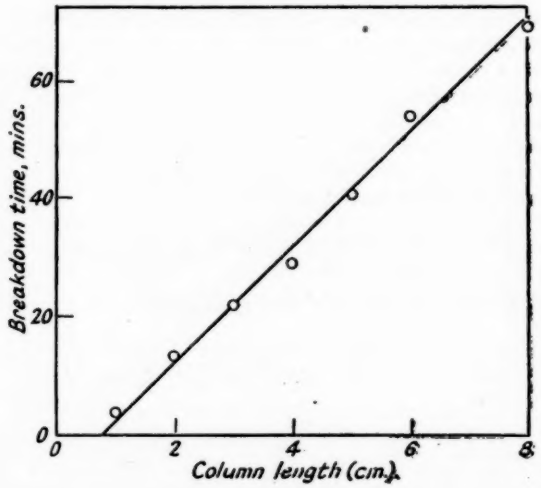


Results.—Effect of flow rate. The effect of flow rate on breakdown volume was determined for columns of different lengths. It was found that a plot of Y against F does not give the linear relation required by equation (3). A plot of Y against $F^{\frac{1}{2}}$ (Fig. 1) gives a close approximation to the linear relation required by equation (5). It is therefore clear that rate of diffusion is important.

Effect of column length. The relationship between breakdown time and column length of equation (2) holds very precisely for the range investigated, *i.e.*, 1—8 cm. (see Fig. 2). In this and the following

FIG. 2.

Effect of column length on breakdown time.



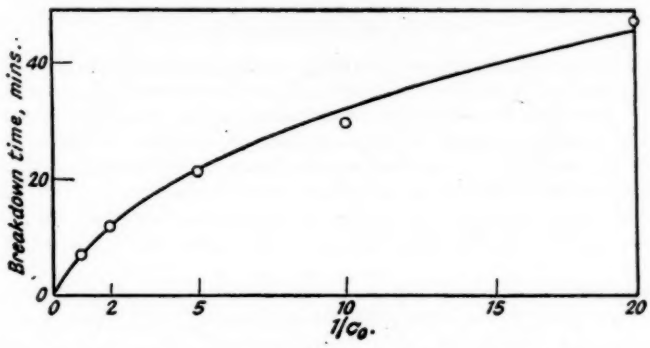
investigations it was found necessary to make slight corrections when the flow rate varied slightly from 2 c.c./min. The correction was made to the breakdown volume on the basis of the curves in Fig. 1, and the breakdown time deduced from the corrected breakdown volume.

Effect of initial concentration of solution. The breakdown times of 4-cm. columns to acetic acid solutions varying in concentration from N to N/20 were determined at a flow rate of 2 c.c./min. The

results are given in Fig. 3, where the breakdown time is plotted against $1/c_0$. The results are in accord with equation (1).

FIG. 3.

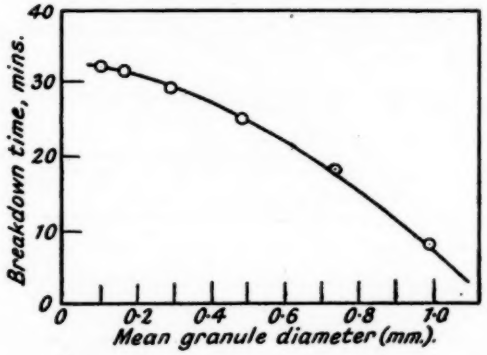
Effect of concentration of acetic acid on breakdown time.



Effect of granule size of charcoal. The breakdown times of 4-cm. columns of charcoal to N/10-acetic acid flowing at 2 c.c./min. were determined for a series of different gradings. According to the equation given in the original theory, the breakdown time should vary linearly with $1/d$, where d is the mean diameter of the granules at a given grading. This relationship is not obeyed. Fig. 4, however, shows

FIG. 4.

Effect of granule size on breakdown time.



that the results follow a linear relationship between breakdown time and mean granule diameter (as required by Equation 6), though there is some falling off from linearity at fine gradings.

Discussion.—The results given above show that the general theory (*J.*, 1946, 918) is largely applicable to the adsorption by charcoal of acetic acid from aqueous solution. One important exception has been found in the effect of grading on breakdown time. In the discussion on gases it was stated that the behaviour of any gas–charcoal pair is determined by the two quantities k and N_0. It was assumed for that case that k is independent of granule size and that therefore any effect of grading on breakdown time must be due to changes in N_0. This seems unlikely in the present case, since the area of adsorbing surface per g. suffers no appreciable change with granule size. It seems more probable that it is k, rather than N_0, which changes with grading, on account of alteration in the accessibility of the adsorbing surface.

The possibility of the general application of the theory to all solutes cannot be assessed until further data are available. The present results suggest: (*a*) That under similar conditions, all charcoals are likely to behave qualitatively in the same way as that used in this work; there will, however, be considerable quantitative differences in rates of adsorption, diffusion and so on, according to the degree of activation of the charcoal and the limitations imposed by the nature of the raw material. (*b*) That a change in the solute is also likely to cause quantitative changes in the rates; if these changes are great, they may result in what appears to be a qualitative change—thus the breakdown time of charcoal columns to methylene-blue is not directly proportional to the square root of the flow rate (C. J. Danby, private communication). The linear relationship given

by acetic acid indicates that diffusion is the rate-determining step in the adsorption process. This is not so for methylene-blue, for which it is probable that several other factors such as actual rate of adsorption on the surface are involved, combining to give a complex relation between breakdown time and flow rate. These may depend upon the size and shape of the molecule of adsorbate.

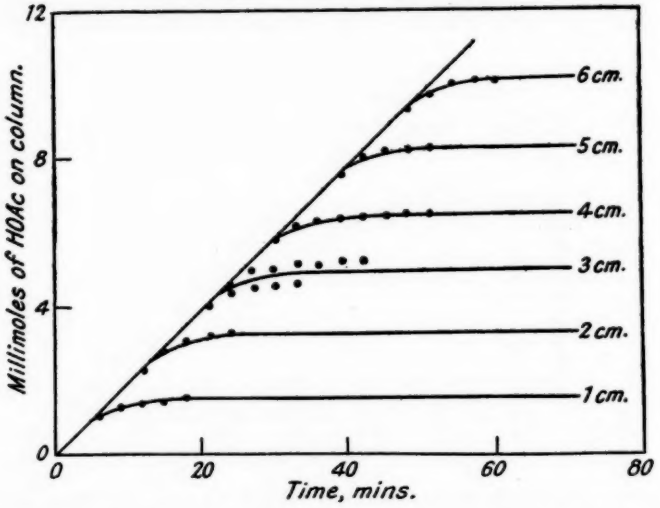
Distribution Phenomena.

We have so far considered mainly the changes occurring in the streams carrying the adsorbate; we shall now consider the complementary problem of the way in which the adsorbate is distributed along the column: first, the development of the distribution during adsorption and then the corresponding changes taking place in subsequent desorption when a stream of pure solvent flows into the column. We shall use the second approximation (see above), which depends on the assumption that equilibrium between the adsorbate in the stream and on the charcoal is attained rapidly relative to the rate of flow of the stream. With this assumption, the only experimental datum required for calculating the distribution of any adsorbate at a given flow rate is its adsorption isotherm.

A rigid mathematical treatment would use the continuous process of the calculus. The approximate method follows this as closely as possible by considering the events taking place in a

FIG. 5.

Adsorption of acetic acid on charcoal columns.



succession of small elements of the column in successive small time intervals. The method of calculation is described in detail by Barrow, Danby, Davoud, Hinshelwood, and Staveley (*loc. cit.*). We shall consider the following cases: (*a*) the adsorption of acetic acid on a charcoal column, (*b*) desorption of acetic acid from an initially completely saturated column, (*c*) desorption of acetic acid from a column of which the first section only (*viz.*, one-third of the column) is initially saturated.

In adsorption the advance of the wave-front proceeds at a fairly uniform rate throughout and a standard time interval can therefore be used for the whole of the calculation. Desorption, however, although initially fast, becomes much slower as the process continues. The time intervals must therefore be small at the beginning of the calculation, but may become much longer towards the end.

Experimental.—A steam-activated nutshell charcoal, graded 80—100 B.S.S. mesh, was used in a column 6 cm. long and of 16 mm. diameter. A standard flow rate of 2 c.c./min. was used throughout. In the adsorption experiments, N/10-acetic acid was passed through the column.

For the desorption experiments, a supply of the charcoal was shaken with N-acetic acid (1 g. of charcoal with 25 c.c. of solution) until equilibrium was established. The supply was used for making up the columns for experiment (*b*) and for the first 2 cm. of the columns for experiment (*c*), the remainder of which were formed from untreated charcoal. Desorption was carried out with distilled water.

The experimental determination of the distribution is based on the principle that, if a solution is passed for a given time through a column 1 cm. long, then the amount of acetic acid adsorbed on this

column is identical with the amount adsorbed on the first cm. of any longer column under the same conditions. Thus, for a 2-cm. column, the difference between this amount and the total amount adsorbed by the 2-cm. column is the amount on the second cm. length. Repetition of this process for columns 1, 2, 3, 4, 5, and 6 cm. long gives the distribution for the whole column.

FIG. 6.

Distribution of acetic acid on a charcoal column during adsorption.

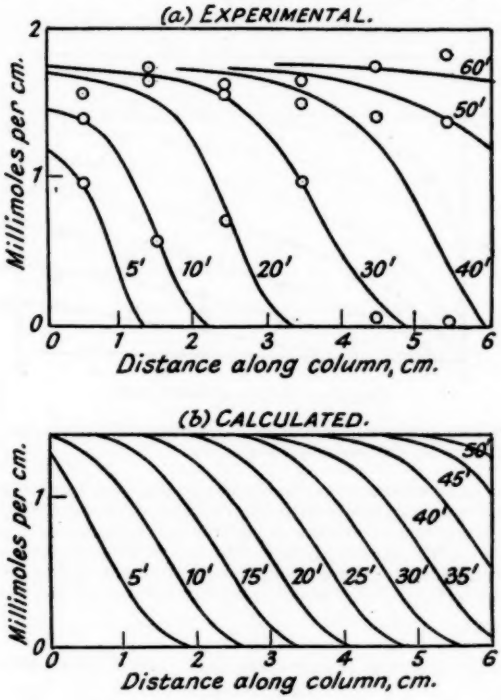


FIG. 7.

Desorption of acetic acid from charcoal columns.

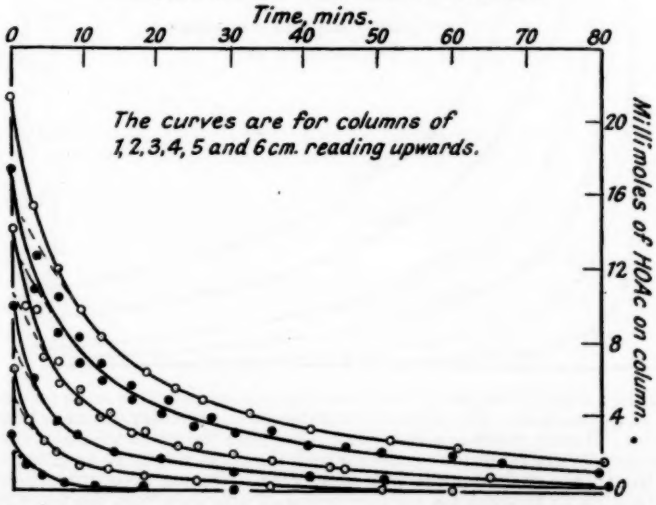


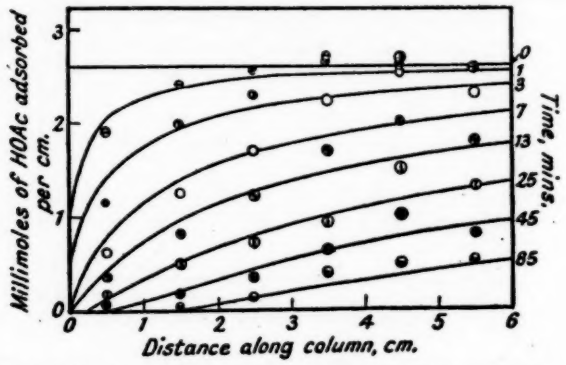
Fig. 5 shows the curves of total adsorption against time for the six columns. At any instant the vertical heights between the adjacent curves give the amounts of acetic acid adsorbed on the corresponding sections of the standard column. In the distribution curves (Fig. 6*a*), the points have been plotted, as a first approximation, at the centre of the respective cm. lengths.

The curves in Fig. 5 were obtained by titrating the acid in the effluent from the columns after successive small time intervals. The difference between the amounts appearing in the effluent and the amount entering the column is the amount adsorbed on the column during the interval.

In desorption, the same technique applies. Titration of the effluent gives the amount desorbed in any given time interval. From the amount initially present on the charcoal, the amount remaining can be deduced. This gives the curves of Figs. 7 and 9, from which the distributions are obtained.

FIG. 8.

Distribution of acetic acid during desorption: initial distribution uniform.

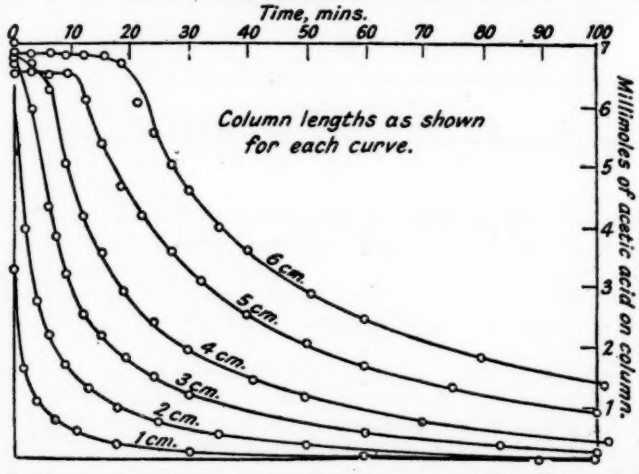


The points represent "corrected" experimental determinations. The curves are calculated distributions.

In the case of desorption, a correction must be applied to the experimentally determined distributions before they can be compared quantitatively with the calculated distributions. It arises because, after adsorption has taken place, the column contains, in addition to the acetic acid adsorbed on the surface, an extra amount present in the carrier stream which is retained in the pore spaces of the charcoal granules. The problem does not arise in the case of gases, since the usual concentrations are so small that the amount held in the carrier stream is negligible. For acetic acid, however, a much higher molecular concentration

FIG. 9.

Desorption of acetic acid from charcoal columns. Initial distribution non-uniform.



has been used and the amount retained in the pores (but not adsorbed) is an appreciable fraction of the amount adsorbed on the surface. The isotherm from which the calculations have been made refers only to surface adsorption. Hence there is a discrepancy between the calculated and the observed results. During adsorption the effect of retention of adsorbate in the pores is only to heighten the experimentally determined wave front; the shape is otherwise unaffected. During desorption, however, the effect is more marked. It is difficult to allow for the desorption of solutions from the pores in the calculations, but an empirical correction has been applied to the experimental results of Fig. 7. The dotted curves are produced back to a value at zero time corresponding to surface adsorption only. These curves give the experimental distributions of surface-adsorbed acetic acid, which are in very close agreement with the calculated distributions (Fig. 8). A similar correction would be more difficult to apply in the case of

non-uniform initial distribution, but the effective agreement between the calculated and uncorrected experimental distributions is sufficiently clear from Fig. 10.

FIG. 10.

Distribution of acetic acid during desorption. Initial distribution non-uniform.

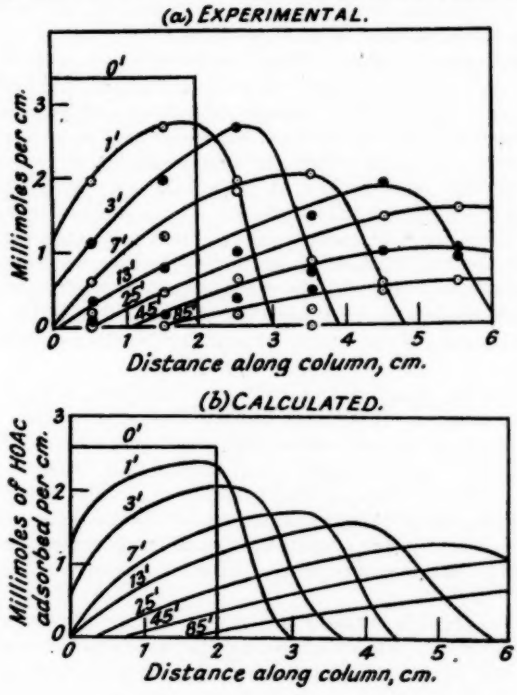
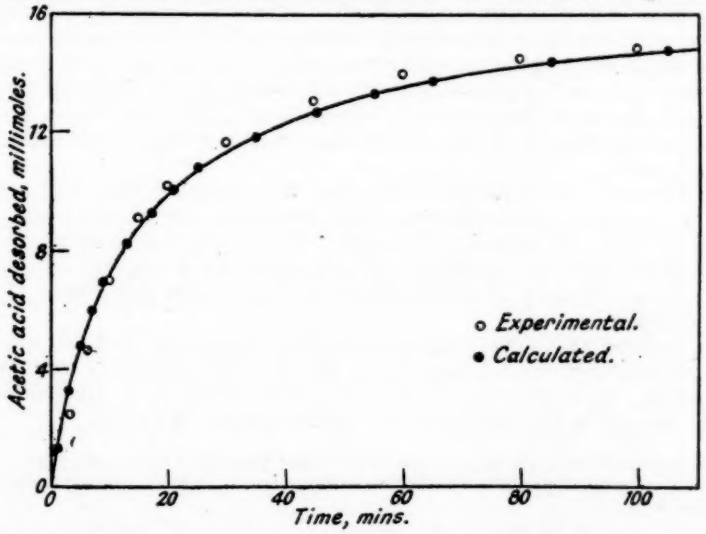


FIG. 11.

Desorption of acetic acid from a charcoal column : total acetic acid in effluent.



The calculations also give the total amount of acetic acid appearing in the effluent as a function of time. The calculated values are compared with the corrected experimental results in Fig. 11.

The experimentally determined isotherm for the charcoal used in this work can be represented approximately by the Langmuir equation

$$x/m = 2c/(1 + 11{\cdot}6c)$$

where x/m is the adsorption of acetic acid in g. of acid per g. of charcoal, and c is the normality of the solution.

Discussion.—The agreement between the experimental and the calculated results is very close, not only for simple adsorption and desorption, but also for the more complex case of the adsorption–desorption cycle which occurs in desorption from a non-uniform initial distribution of adsorbate. We can conclude that both adsorption and desorption are very fast processes. Taken in conjunction with the conclusion of the first part of this paper that diffusion is probably the rate-determining step in these processes, the evidence suggests that adsorption and desorption take place as fast as the molecules can be brought to or removed from the charcoal surface. In gaseous adsorption, where the flow rate which has been used is so fast (the linear flow rate is 250 cm./min.) that contact time of the molecules with the surface is very small, the establishment of equilibrium at the surface must be almost instantaneous. With solutions, a much slower linear flow-rate of about 1 cm./min. has been used. There is consequently no direct evidence of the rate of the surface reactions. It seems probable, however, that these may still be fast and that the rate of removal of solute from solutions is governed by rate of diffusion to the surface.

Of the combined processes of diffusion to the surface and reaction there, during adsorption, and of the corresponding processes for desorption, it can certainly be said that the flow rates used in this work have allowed sufficient time for equilibrium to be almost completely established throughout.

In these circumstances the shape of the isotherm completely determines the distributions of the adsorbate along the column. The most important feature of the isotherm is that a high degree of adsorption is obtained at low concentrations of acetic acid. Hence the wave-front in adsorption is very sharp and nearly vertical, *i.e.*, the column becomes nearly saturated before any adsorbate appears in the effluent. When breakdown occurs, however, the effluent concentration rises very rapidly to that of the ingoing stream. It is a direct consequence of the shape of the isotherm in the region near the origin, which has the practical result that the charcoal is very efficient in removing small traces of acetic acid from aqueous solution and remains so until it is nearly saturated.

In desorption the wave-front is initially very steep, but rapidly becomes much flatter. This again is because large amounts of adsorbate can remain on the surface and yet give rise to only a small concentration in the stream. Consequently, the whole column *tends* towards a common level of adsorption at which the rate of desorption is very slow, *i.e.*, the wave-front tends to become very flat.

We conclude that the theories of adsorption developed for the general case apply very well to adsorption of acetic acid from aqueous solution. There is a strong similarity between the adsorption characteristics of acetic acid and of carbon tetrachloride vapour (which is typical of gases which undergo simple physical adsorption only). This similarity is possible because, of the molecular and ionic species present in acetic acid solutions, only the undissociated molecules are adsorbed, *i.e.*, the same phenomenon of physical molecular adsorption is shown by both substances. The isotherms are of very similar shape. The fundamental processes appear to be the same in both media.

For both substances, the conditions controlling the breakdown time indicate that the rate-determining step in this process is diffusion of the adsorbate to the internal surface of the granule. The reaction at the surface seems to be very rapid. Provided that the flow rate be slow compared with the rate of diffusion, the conditions of adsorption are effectively those of equilibrium. Consequently, the distribution of the adsorbate on the column depends on the shape of the adsorption isotherm only.

The results outlined above apply to aqueous solutions of acetic acid and would be expected to apply to other solutes in which diffusion to or from the charcoal surface is the rate-determining step in the process. It is unlikely, however, that they will apply to all solutes, since rates of adsorption and desorption for larger molecules are sometimes very slow.

This paper is published with the permission of the Chief Scientific Officer, Ministry of Supply.
The author is very grateful to Professor Sir Cyril Hinshelwood, F.R.S., for his helpful criticism during the course of this work.

PHYSICAL CHEMISTRY LABORATORY, OXFORD. [*Received, June 16th.* 1947.]

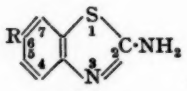
299. *The Ultra-violet Absorption Spectra of Some 2-Aminobenzthiazole and 2-Imino-3-methyl-2 : 3-dihydrobenzthiazole Derivatives.*

By J. R. Edisbury, R. F. Hunter, and A. D. Scott.

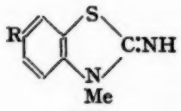
The ultra-violet absorption spectra of a number of 2-amino- and 2-imino-3-methyl-2 : 3 dihydro-benzthiazoles have been determined. There is a barely significant shift in absorption maximum towards the region of longer wave-length on replacing hydrogen by fluorine in the 6-position in 2-aminobenzthiazole, a marked shift when fluorine is replaced by chlorine, no further displacement when chlorine is replaced by bromine, and another small shift when iodine replaces bromine. Methylation of the aromatic nucleus causes small shifts towards the region of longer wave-length and a similar effect occurs on alkylation of the 2-amino-group.

The 2-imino-3-methyl-2 : 3-dihydrobenzthiazoles show two absorption bands, and the shift of that of higher wave-length which occurs on substitution of hydrogen by bromine in the 6-position is identical with that which occurs in the 2-aminobenzthiazole system.

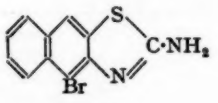
In an investigation of the effect of substituents on the ultra-violet absorption of aromatic and aromatic heterocyclic compounds, the absorption spectra of a number of 2-aminobenzthiazole (I) and 2-imino-3-methyl-2 : 3-dihydrobenzthiazole (II) derivatives, and of 4-bromo-2-amino-



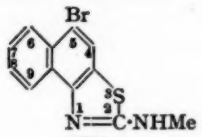
(I.)



(II.)



(III.)



(IV.)

ββ′-naphthathiazole (III) and 5-bromo-2-methylamino-β-naphthathiazole (IV) were examined. The results are summarised in the table below :

Compound.	Abs. maxima, mμ.	ε, max.
2-Aminobenzthiazole	263	12,900
6-Fluoro-2-aminobenzthiazole	264	11,400
6-Chloro-2-aminobenzthiazole	272	20,300
6-Bromo-2-aminobenzthiazole	272	15,500
6-Iodo-2-aminobenzthiazole	276	20,500
6-Chloro-4-bromo-2-methylaminobenzthiazole	280 and 233	19,000 and 38,500
2-Amino-4 : 6-dimethylbenzthiazole	269	15,900
2-*iso*Butylamino-4-methylbenzthiazole	272	13,250
2-Dimethylamino-4-methylbenzthiazole	283 and 230	24,000 and 12,900
2-Ethylamino-6-methylbenzthiazole	271	20,100
4-Bromo-2-amino-6-methylbenzthiazole	269	14,500
4-Bromo-2-*iso*butylamino-6-methylbenzthiazole	278	15,100
2-Imino-3-methyl-2 : 3-dihydrobenzthiazole	300 and 265	4850 and 8200
6-Bromo-2-imino-3-methyl-2 : 3-dihydrobenzthiazole	309 and 270	4900 and 9400
2-Imino-6-ethoxy-3-methyl-2 : 3-dihydrobenzthiazole	327 and 270	5650 and 7300
4-Bromo-2-amino-ββ′-naphthathiazole	265	48,800
5-Bromo-2-methylamino-β-naphthathiazole	258 and 340	41,500 and 10,800

In the 6-halogeno-2-aminobenzthiazoles, there is a barely significant shift towards the region of longer wave-length on passing from hydrogen to fluorine, a marked shift from fluorine to chlorine, no further displacement when chlorine is replaced by bromine, and finally another small shift when iodine replaces bromine (Fig. 1). This phenomenon is in general agreement with many of the physical properties of halogen-substituted compounds.

It is noteworthy that replacement of the 4-methyl group by bromine in 2-amino-4 : 6-dimethylbenzthiazole has practically no effect on the intensity of the absorption band whose maximum remains at the same wave-length (269 mμ) in 4-bromo-2-amino-6-methylbenzthiazole.

Methylation in the aromatic nucleus of the benzthiazole system causes a small shift of absorption maximum towards the region of longer wave-length. A similar effect occurs on alkylation of the 2-amino-group which increases with size of the alkyl substituent (cf. Burawoy, *J.*, 1939, 1177).

The appearance of the two absorption maxima in the 2-imino-3-methyl-2 : 3-dihydrobenzthiazoles and in 2-dimethylamino-4-methylbenzthiazole in all of which the semi-cyclic triad system [H]N·C:N ⇌ N:C·N[H] has been rendered static by substitution of "mobile" hydrogen by methyl (Hunter, *J.*, 1930, 125; Hunter and Jones, *ibid.*, p. 2190) (Fig. 2) is also interesting.

FIG. 1.

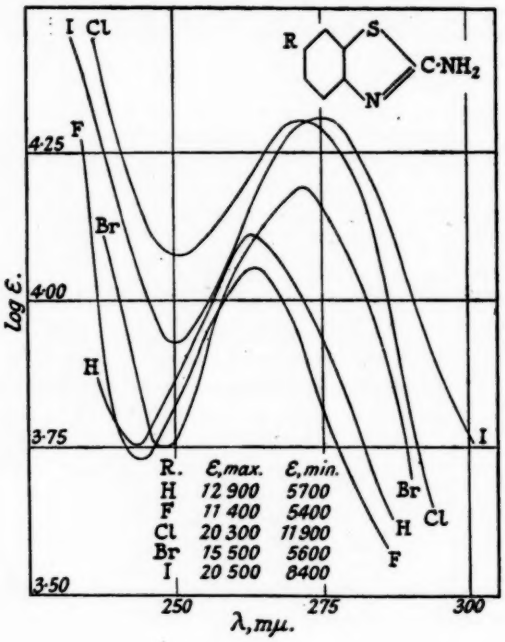
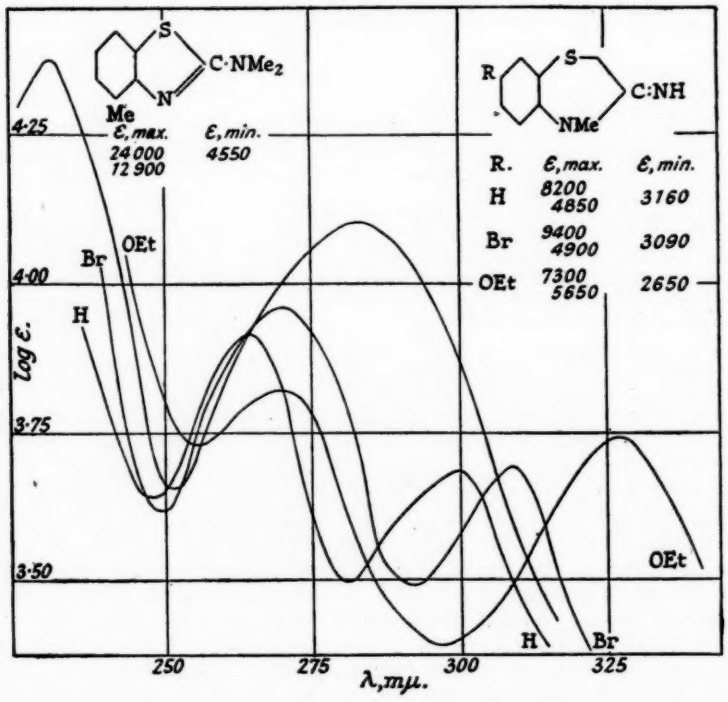


FIG. 2.



The shift of the absorption band of higher wave-length which occurs on substitution of hydrogen by bromine in the 6-position in 2-imino-3-methyl-2 : 3-dihydrobenzthiazole (9 mμ) is identical with that which occurs in the 2-aminobenzthiazole system.

The high absorption of 4-bromo-2-amino-ββ′-naphthathiazole and of 5-bromo-2-methylamino-β-naphthathiazole is evidently connected with the presence of the additional fused aromatic ring, the systems being essentially diheteroanthracene and phenanthrene respectively.

EXPERIMENTAL.

The specimens measured were either those described in the following references or prepared as described in these papers. Benzthiazoles: 2-amino- (Hunter, *J.*, 1926, 1385); 6-fluoro-2-amino- (Dyson, Hunter, Jones, and Styles *J. Indian Chem. Soc.*, 1931, **8**, 147); 6-chloro-2-amino- (Dyson, Hunter, and Morris, *J.*, 1927, 1186); 6-bromo-2-amino- (Hunter, *loc. cit.*); 6-iodo-2-amino- (Dyson, Hunter, Jones, and Styles, *loc. cit.*); 6-chloro-4-bromo-2-methylamino- (Dyson, Hunter, Jones, and Styles, *loc. cit.*); 2-amino-4 : 6-dimethyl- (Hunter and Pride, *J.*, 1929, 943); 2-*iso*butylamino-3-methyl- (Dyson, Hunter, Jones, and Styles, *loc. cit.*); 2-dimethylamino-4-methyl- (Hunter and Styles, *J.*, 1927, 1209); 2-ethylamino-6-methyl- (Dyson, Hunter, Jones, and Styles, *loc. cit.*); 4-bromo-2-amino-6-methyl- (Hunter, *J.*, 1926, 1385); 4-bromo-2-*iso*butylamino-6-methyl- (Dyson, Hunter, Jones, and Styles, *loc. cit.*); 2-imino-3-methyl-2 : 3-dihydro- (Hunter, *J.*, 1926, 1385); 6-bromo-2-imino-3-methyl-2 : 3-dihydro- (Hunter, *J.*, 1930, 125); 2-imino-6-ethoxy-3-methyl-2 : 3-dihydro- (Hunter and Jones, *J.*, 1930, 2190). 4-Bromo-2-amino-ββ′-naphthathiazole (Hunter and Jones, *J.*, 1930, 941). 5-Bromo-2-methylamino-β-naphthathiazole (Chiragh Hasan and Hunter, *J. Indian Chem. Soc.*, 1933, **10**, 82).

The compounds were dissolved in absolute alcohol and diluted as necessary.

Measurements of ultra-violet light absorption were made on a Hilger (E 498) quartz medium spectrograph with Spekker photometer, using a condensed spark between tungsten-steel electrodes. Kodak Type IV 0 plates were used in most instances for the purpose of providing adequate photographic contrast.

RESEARCH DEPARTMENT, LEVER BROTHERS AND UNILEVER LTD.,
PORT SUNLIGHT. [*Received, August 27th*, 1947.]

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300. *The Condensation of* 2 : 3-*Dimethyl*-1 : 3-*butadiene and* p-*Benzoquinone.*

By M. LORA-TAMAYO and J. L. LEON.

The paper describes a preliminary examination, using 2 : 3-dimethylbutadiene, of conditions for the quantitative estimation of conjugated butadienoid systems by combination with *p*-benzoquinone. At ordinary temperatures these compounds combine in 1 : 1 ratio. The product, m. p. 116°, on heating passes into an isomeride, m. p. 235°. Only the original product gives an oxime, but both isomerides yield the same acetyl derivative. The original product is therefore regarded as the normal adduct, and the higher-melting compound as the isomeric quinol derivative. Morgan and Coulson have already reported the addition of 2 : 3-dimethylbutadiene to *p*-benzoquinone in 2 : 1 ratio at higher temperatures. It is now shown that not only addition in 1 : 1 ratio under the conditions now described, but also addition in 2 : 1 ratio under Morgan and Coulson's conditions, can be made the basis of a method of estimating 2 : 3-dimethylbutadiene by measurement of uptake of *p*-benzoquinone.

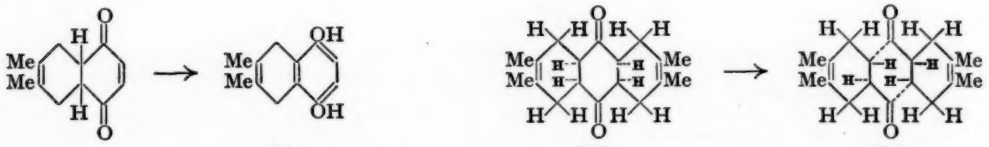
THE Diels condensation between conjugated dienes and maleic anhydride is used for the estimation of the former by volumetric determination of the residual maleic anhydride (*Fette und Seife*, 1936, **43**, 93). Developed by Kaufmann under the name of " dienometry ", it has been introduced especially in the analysis of fats and of essential oils, a new constant having been established for them, the " diene index " (*Ber.*, 1937, **70**, 903, 905).

But these analytical applications are unfavourably influenced by phenomena of isomerisation, and, above all, by the possibility of the maleic anhydride's reacting with other unconjugated and even simple ethylene compounds by a process of addition involving the splitting of a C–H bond (Alder, *Ber.*, 1943, **76**, 27; Lora, *Rev. Acad. Farm. Madrid*, 1944, **10**, 1).

Consequently, while we were engaged on other work on condensations with *p*-benzoquinone (Lora and Fernandez, *Anal. Fís. Quím.*, 1944, **40**, 1178; 1946, **42**, 1183), we undertook the investigation of the quantitative determination of conjugated systems using this philodiene. This presupposed in every case a knowledge of the conditions for obtaining the addition product, in order to fix those most suitable for the quantitative application of the reaction. The estimation of *cyclo*pentadiene by its reaction with benzoquinone has been described by Wasserman (*J.*, 1936, 1032).

Among the compounds investigated, 2 : 3-dimethylbutadiene gave special results in its condensation with *p*-benzoquinone. Morgan and Coulson (*J.*, 1931, 2323) investigated it in alcoholic solution in a sealed tube at 97° for 5 hours, and obtained a product whose melting point was 202—203°, and whose formula corresponded to the addition of two molecules of diene to one of *p*-benzoquinone. Working at ordinary temperatures, we found that the alcoholic solution,

treated with water, precipitated some crystalline needles of a pale yellow colour and m. p. 116°, whose analysis corresponded to a *compound* of one molecule of 2 : 3-dimethylbutadiene and one of *p*-benzoquinone.



(I.) (II.) (III.) (IV.)

Morgan and Coulson's product (m. p. 202—203°), after melting, solidified and melted again at 304°. Ours (m. p. 116°) after melting solidifies at 185° and finally melts at 234°.

In both cases the new products are isomeric with their precursors: a similar phenomenon has been reported for adducts of butadiene and isoprene (Diels and Alder, *Ber.*, 1929, **62**, 2337). Morgan and Coulson, in view of the fact that their isomer is not capable of acetylation, did not consider it as a derivative of quinol, but rather as a stereoisomer (III and IV) analogous to that of bisisoprene–quinone (Diels and Alder, *loc. cit.*, p. 2366), probably originating through an intermediate form of quinol.

In our case both products acetylate and give the same *diacetyl* derivative (m. p. 134°), but only that of lower melting point gives an *oxime*. This shows that the isomerism leads to the quinol *form* (II), as is found in the transformation of monobutadiene–quinone (Diels and Alder, *loc. cit.*, p. 2362). D.R.-PP. 324,661 and 521,621 (*Chem. Zentr.*, 1930, II, 809; 1931, II, 1758) refer to these products of 1 : 1 addition of dimethylbutadiene and *p*-benzoquinone.

Neither of the two products (I and II) adds 2 : 3-dimethylbutadiene under Morgan and Coulson's conditions. With regard to (II), this was due to lack of the appropriate conjugation, and with regard to (I) it was because (I) isomerises to (II). The isomerisation can also be produced by the action of hydrochloric acid.

With regard to the analytical applications of the condensation, these were tried, in the first place in the cold, the basic reaction in this case being the formation of the 1 : 1-addition product. A procedure was also studied which involved heating the reactants in sealed tubes at 100° for 5 hours, calculations being then made on the assumption that addition had taken place in the 2 : 1 ratio. In each case the operation was carried out with excess of *p*-benzoquinone in alcoholic solution. The residual *p*-benzoquinone was estimated according to Willstätter and Majina's method (*Ber.*, 1910, **43**, 1171), the iodine liberated from an acid solution of potassium iodide being determined. The combined quinone was deduced as a difference between the initial amount, given by a blank determination, and the measured excess. As an example we give some results in the experimental section.

Experimental.

Condensation of 2 : 3-*Dimethylbutadiene and* p-*Benzoquinone.* 6 : 7-*Dimethyltetrahydro-α-naphthaquinone.*—The dimethylbutadiene used was prepared from pinacol by reduction with hydrobromic acid (Kyriakides, *J. Amer. Chem. Soc.*, 1914, **36**, 991). An alcoholic solution of *p*-benzoquinone (10 g.) and dimethylbutadiene (4 g.) was kept at room temperature for 4 hours. Water was then added. The pale yellow needles were filtered off and washed well with water to eliminate the excess of quinone. After being dissolved in alcohol and precipitated with water, the product (8·5 g.) was very pale yellow and had m. p. 116° (I). When heated above its melting point, it solidified between 186° and 190° to a white *product* which melted at 235° (II). When the preparation was repeated with different concentrations of benzoquinone and dimethylbutadiene, the result obtained was always the same (Found: C, 75·7; H, 7·7. $C_{12}H_{14}O_2$ requires C, 75·8; H, 7·4%).

Isomerisation with Hydrochloric Acid.—The product, m. p. 116° (2 g.), was dissolved in alcohol, a few drops of concentrated hydrochloric acid were added, and the mixture was boiled under reflux for 5—10 minutes. To the cooled solution, which had lost its yellow colour, water was added, and a white product was thus precipitated. This was collected, washed with water, and purified as described above. Crystallised from chlorobenzene, it had m. p. 235° (Found: C, 75·8; H, 7·7%).

1 : 4-*Diacetyl*-6 : 7-*dimethyl*-5 : 8-*dihydro*-1 : 4-*naphthaquinol.*—In separate flasks 1·5 g. of (I) and (II) were heated under a reflux for 2 hours with 10 g. of acetic anhydride. On cooling, beautiful crystals of the *diacetyl* compound separated, which on recrystallisation from alcohol gave white needles, m. p. 134° in both cases [Found: (I) C, 69·9; H, 6·5. (II) C, 69·8; H, 6·4. $C_{16}H_{18}O_4$ requires C, 70·1; H, 6·5%].

6 : 7-*Dimethyl*-5 : 8 : 9 : 10-*tetrahydro*-1 : 4-*naphthaquinone Dioxime.*—The test was carried out simultaneously with (I) and (II) in alcoholic solution using 1 g. of either, 1 g. of hydroxylamine hydrochloride, and the appropriate quantity of sodium hydroxide. In the case of (I), after ½ hour's heating, the *oxime* was formed, and was then recrystallised from alcohol. It had m. p. 234° (Found: N, 12·5. $C_{12}H_{16}O_2N_2$ requires N, 12·7%). In the case of (II), the starting product was always recovered unchanged even though heating was prolonged to 3 hours.

Reactions of (I) *and* (II) *with Dimethylbutadiene.*—An attempt was made to condense (I) and (II) with another molecule of dimethylbutadiene. Morgan and Coulson's method (*loc. cit.*) was followed, an alcoholic solution (50 c.c.) of (I) or (II) (0·6 g.) being heated with dimethylbutadiene (0·4 g.) in a sealed tube at 90—100° for 6 hours. In both cases the product was precipitated with water, collected, and washed. Purified as usual, and finally recrystallised from monochlorobenzene, the product was (II), m. p. 235°.

Analytical Application.—We used dimethylbutadiene, purified by distillation, and *p*-benzoquinone, purified by recrystallisation from alcohol. The operations were always carried out in alcoholic solution, and for each determination a blank was done using *p*-benzoquinone alone in the same quantity and at the same dilution. Two different types of method were used. In the one the reaction was allowed to go in the cold for 24 hours, as for the preparation of 6 : 7-dimethyltetrahydro-1 : 4-naphthaquinone, in which the ratio of diene to philodiene was 1 : 1. The results are in Table I. The second group of tests were carried out in sealed tubes at 90—100° in order to obtain the diene–philodiene ratio of 2 : 1, as was done by Coulson and Morgan (*loc. cit.*). The results are given in Table II.

TABLE I.

Diene taken.	Quinone added.	Quinone condensed.	Diene found.	Error, %.
0·2691	0·5016	0·3473	0·2657	1·3
0·2691	0·5016	0·3475	0·2639	1·9
0·1345	0·2142	0·1739	0·1325	1·4
0·1345	0·2142	0·1743	0·1326	1·4

TABLE II.

Diene taken.	Quinone added.	Quinone condensed.	Diene found.	Error, %.
0·2691	0·1908	0·1737	0·2638	1·9
0·2691	0·1908	0·1740	0·2642	1·5
0·1345	0·1825	0·0869	0·1329	1·2
0·1345	0·1825	0·0872	0·1328	1·2

In both cases the technique employed was the same in every detail. At the end of the period of reaction the product formed was precipitated with water, collected, and washed abundantly, the filtrate being collected quantitatively in a graduated flask. The volume was made up with water and portions were taken for the iodometric determination of benzoquinone. For this the volume taken was diluted to 150 ml. Excess of potassium iodide was added, and the solution acidified with hydrogen chloride and left in the dark for 10 to 15 minutes so that the liberation of iodine by the quinone should proceed to completion. Then the free iodine was evaluated with thiosulphate using starch indicator. At the same time a blank was carried out with *p*-benzoquinone. From the figures for quinone found in the blank, the excess found in the tests with dimethylbutadiene was subtracted, the value for the condensed quinone being thus obtained.

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ORGANIC CHEMISTRY SECTION, MADRID.

[*Received, November 13th,* 1947.]

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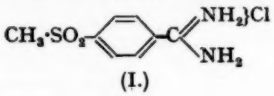
301. *Chemotherapeutic Agents of the Sulphone Type. Part III. Effect of Homologation on the Antibacterial Activity of* p-*Methylsulphonylbenzamidine and* p-*Methylsulphonylbenzylamine Hydrochlorides.*

By H. S. FORREST, A. T. FULLER, and JAMES WALKER.

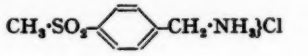
Separating the functional groups from the benzene ring in *p*-methylsulphonyl-benzamidine and -benzylamine hydrochlorides by one or more methylene groups is accompanied by a diminution in antibacterial activity *in vitro*.

FOLLOWING the discovery of pronounced antibacterial activity in *p*-methylsulphonyl-benzamidine and -benzylamine hydrochlorides [(I) and (II)] (Evans, Fuller, and Walker, *Lancet*, 1944, *ii*, 523; 1945, *ii*, 336), the effects of altering the orientation of the functional groups and of varying the size of the alkyl radical attached to the sulphonyl group were studied (Fuller, Tonkin, and Walker, *J.*, 1945, 633). The conclusion was reached that, for high antibacterial activity, compounds should conform to type (III), where R may be a small alkyl group, as in (I) and in (II), or the $-NH_2$ group, as in marfanil (III; R = NH_2, R′R″ = H_2) which has a similar antibacterial "spectrum" (Evans, Fuller, and Walker, *locc. cit.*), and R′R″ may be an imino-group, as in (I), or two hydrogen atoms, as in (II) and in marfanil, or even one hydrogen atom and one methyl group. The work described in the present paper was designed to test the validity of the above generalisation by the study of further homologues (IV) of the original parent compounds (I) and (II), the aim being to observe the effect of increasing the separation of the functional groups from the benzene ring. In the previous study this had been limited to the α- and β-phenylethylamine

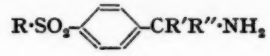
homologues of (II), where diminished antibacterial activity paralleled experience in the marfanil series (Lawrence, *J. Bact.*, 1945, **49**, 149).



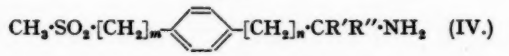
(I.)



(II.)



(III.)



(IV.)

The introduction of a methylene group between the benzene ring and the methylsulphonyl group of (I) and (II) was first undertaken, the essential intermediate, p-*cyanobenzyl methyl sulphone* (V), being obtained in quantitative yield by oxidation of p-*cyanobenzyl methyl sulphide* (VI). p-*Amidinobenzyl methyl sulphone hydrochloride* (VII) was obtained from (V) by the orthodox Pinner technique, while p-(*aminomethyl*)*benzyl methyl sulphone hydrochloride* (VIII) resulted from catalytic hydrogenation of (V) in presence of Raney nickel. Since the original survey had shown *p*-methylthiobenzamidine hydrochloride to have marked antibacterial activity, the homologous p-*amidinobenzyl methyl sulphide hydrochloride* (IX) was prepared from the appropriate nitrile (VI) in the usual way. Access to the corresponding primary amine was obtained only with difficulty. As was expected from the presence of unshared electrons on the sulphur atom, the nitrile (VI) poisoned the catalyst when catalytic hydrogenation was attempted (cf. Maxted, *J.*, 1945, 204; Deem and Kaveckis, *Ind. Eng. Chem.*, 1941, **33**, 1373), and the product consisted of a mixture of the unchanged starting material and *p*-tolunitrile, as (VI), being a benzyl thioether, was particularly readily susceptible to hydrogenolysis in the presence of Raney nickel (Bougault, Cattelain, and Chabrier, *Bull. Soc. chim.*, 1940, **7**, 781; Mozingo, Wolf, Harris, and Folkers, *J. Amer. Chem. Soc.*, 1943, **65**, 1013). Hydrogenolysis also took place when sodium and alcohol reduction was attempted, the only basic product isolated being *p*-tolubenzylamine. A small yield of p-(*aminomethyl*)*benzyl methyl sulphide hydrochloride* (X) was, however, obtained by reducing (VI) with chromous acetate and alcoholic alkali (cf. Graf, *J. pr. Chem.*, 1934, **140**, 39; 1936, **146**, 88). Further homologation at this position in the molecule was not undertaken, attention being directed to compounds homologated in the alternative position (IV; $m = 0$, $n = 1$—3). When the Willgerodt reaction (Willgerodt and Merk, *J. pr. Chem.*, 1902, **80**, 192; Fieser and Kilmer, *J. Amer. Chem. Soc.*, 1940, **62**, 1354) was applied to *p*-methylsulphonylacetophenone, only a poor yield of p-*methylsulphonylphenylacetamide* (XI) resulted, and a much better yield of p-*methylsulphonylphenylthioacetmorpholide* (XII) resulted by using morpholine in Kindler and Li's process (*Ber.*, 1941, **74**, 321), which was recognised by Schwenk and Bloch (*J. Amer. Chem. Soc.*, 1942, **64**, 3051) as fundamentally a variant of the Willgerodt reaction. Hydrolysis of (XII) to p-*methylsulphonylphenylacetic acid* then gave access to (XI) in convenient amounts, and dehydration of (XI) afforded p-*methylsulphonylphenylacetonitrile*, from which p-*methylsulphonylphenylacetamidine hydrochloride* (XIII) was obtained. The corresponding amine (XIV) was included in the earlier survey (Fuller, Tonkin, and Walker, *loc. cit.*), being obtained by the Curtius–Naegeli degradation of β-*p*-methylsulphonylphenylpropionic acid. An attempt to apply the reaction between diazonium salts and acrylonitrile (Koelsch, *J. Amer. Chem. Soc.*, 1943, **65**, 57; Koelsch and Boekelheide, *ibid.*, 1944, **66**, 412), using diazotised *p*-methylsulphonylaniline, was unsuccessful in providing access to β-p-*methylsulphonylphenylpropionitrile* (XV), which was obtained instead by dehydration of β-p-*methylsulphonylphenylpropionamide*. On amidine formation and catalytic hydrogenation respectively, (XV) afforded β-p-*methylsulphonylphenylpropionamidine hydrochloride* (XVI) and γ-p-*methylsulphonylphenyl*-n-*propylamine hydrochloride* (XVII). Further homologation was accomplished by converting β-*p*-methylsulphonylphenylpropionic acid through the acid chloride into the *diazomethyl ketone* and submitting the latter to the Arndt–Eistert reaction. Dehydration of the resulting γ-p-*methylsulphonylphenyl*-n-*butyramide* afforded the *nitrile* required for the preparation of γ-p-*methylsulphonylphenyl*-n-*butyramidine hydrochloride* (XVIII) and δ-p-*methylsulphonylphenyl*-n-*butylamine hydrochloride* (XIX), by amidine formation and catalytic hydrogenation respectively. As an alternative route to the compounds described above and one which would have been applicable to the further extension of the series if such had been warranted by the biological results, the preparation of *ethyl* p-*methylsulphonylbenzoylacetate* (XX) was undertaken, since this ester could be alkylated with ω-halogenated fatty acid esters, or with ω-phthalimidoalkyl halides, to give essential intermediates after ketonic hydrolysis and Clemmensen reduction. The yields of (XX), however, were not promising, and this route was not further examined.

As all the aromatic α-amino-acids of biological importance—phenylalanine, tyrosine, histidine, and tryptophan—are β-substituted derivatives of α-alanine, it appeared desirable to include β-p-*methylsulphonylphenylalanine* (XXI) in this survey, particularly since Schaffer (*Proc. Soc. Exp. Biol. Med.*, 1937, **37**, 648) has claimed that *p*-sulphonamidophenylalanine is superior to sulphanilamide in hæmolytic streptococcal infections in mice. *p*-Methylsulphonylbenzaldehyde was therefore condensed with hippuric acid, and the resulting *2-phenyl-4-p-methylsulphonylbenzylidene-5-oxazolone* afforded (XXI) on reductive hydrolysis with red phosphorus and hydriodic acid.

The results of antibacterial tests *in vitro*, which support the conclusions reached in the previous paper, are recorded in the Table, the cultures and technique employed being those previously described (Fuller, *Biochem. J.*, 1942, **36**, 548; Evans, Fuller, and Walker, *locc. cit.*). For comparative purposes the values found for a few substances described in earlier papers are included.

Compound, X–C$_6$H$_4$–[CH$_2$]$_n$·Y.				Antibacterial activity *in vitro*. Minimal inhibiting concentrations in mg. of drug per 100 c.c. of nutrient broth.		
	X.	*n*.	Y.	*Strep. hæmolyt.*	*Staph. aureus.*	*Bact. coli.*
	(IX)	$CH_3·S·CH_2$	0	$C(NH_2):NH_2\}Cl$	10	100	500
	(VII)	$CH_3·SO_2·CH_2$	0	$C(NH_2):NH_2\}Cl$	100	1000	>1000
*	(I)	$CH_3·SO_2$	0	$C(NH_2):NH_2\}Cl$	0·15	250	250
	(XIII)	$CH_3·SO_2$	1	$C(NH_2):NH_2\}Cl$	0·5	100	750
	(XVI)	$CH_3·SO_2$	2	$C(NH_2):NH_2\}Cl$	50	200	750
	(XVIII)	$CH_3·SO_2$	3	$C(NH_2):NH_2\}Cl$	150	200	500
	(VIII)	$CH_3·SO_2·CH_2$	1	$NH_2\}Cl$	>1000	>1000	>1000
†		$CH_3·SO_2$	0	NH_2	25	750	75
*	(II)	$CH_3·SO_2$	1	$NH_2\}Cl$	1	5	100
*	(XIV)	$CH_3·SO_2$	2	$NH_2\}Cl$	300	1000	2000
	(XVII)	$CH_3·SO_2$	3	$NH_2\}Cl$	30	300	1000
	(XIX)	$CH_3·SO_2$	4	$NH_2\}Cl$	200	1000	1000
	(XXI)	$CH_3·SO_2$	1	$CH(NH_2)·CO_2H$	500	>500	500

* *J.*, 1945, 633. † *J.*, 1945, 630.

The inhibition of growth of hæmolytic streptococci provides the best basis of comparison, being most sensitive to structural variations in the compounds tested. Comparing (VII) with (I), and (VIII) with (II), it is obvious that the insertion of a methylene group between the methylsulphonyl radical and the benzene ring has the most marked effect of all, causing about a thousand-fold reduction in activity against hæmolytic streptococci. Homologation in the alternative position had a less marked effect on activity, there being a gradual decline in activity with the amidines (I), (XIII), (XVI), and (XVIII), the amidine (XIII) being comparable in activity with the original amidine (I). Analogously, *p*-sulphonamidobenzamidine hydrochloride and the homologous *p*-sulphonamidophenylacetamidine hydrochloride are also comparable in antibacterial activity (Fuller, *Biochem. J.*, 1947, **41**, 403), although, of the two, only the former was found to be active in experimental typhus (Andrewes, King, van den Ende, and Walker, *Lancet*, 1944, *i*, 777; Andrewes, King, and Walker, *Proc. Roy. Soc.*, 1946, *B*, **133**, 20), recalling the fact that the antirickettsial activity of *p*-aminobenzoic acid is lost on similar homologation to *p*-aminophenylacetic acid and related compounds (Hamilton, *Proc. Soc. Exp. Biol. Med.*, 1945, **59**, 220). Among the amines (II), (XIV), (XVII), and (XIX) a clearly marked alternation in antibacterial activity was observable. The activity of (XXI) was lower than expected in view of Schaffer's claim (*loc. cit.*), and also in view of the fact that the biosynthesis, or utilisation, of phenylalanine by some micro-organisms is antagonised by certain structural analogues (Beerstecher and Shive, *J. Biol. Chem.*, 1946, **164**, 53; 1947, **167**, 527; *J. Amer. Chem. Soc.*, 1947, **69**, 461; du Vigneaud *et al.*, *J. Biol. Chem.*, 1945, **159**, 385; Dittmer *et al.*, *ibid.*, 1946, **164**, 761), although the levels at which these structural analogues inhibit the relevant micro-organisms are not of the low order encountered in antibacterial compounds.

Experimental.

p-*Cyanobenzyl Methyl Sulphide* (VI).—To a solution of sodium methyl sulphide, obtained by adding 5N-sodium hydroxide solution (16 c.c.) to *S*-methylthiouronium sulphate (11 g.) and collecting the evolved methanethiol in absolute alcohol (30 c.c.) containing sodium ethoxide (from 1·6 g. of sodium), there was added, in the course of about 5 minutes, a hot saturated solution of *p*-cyanobenzyl bromide (14·8 g.) (Case, *J. Amer. Chem. Soc.*, 1925, **47**, 1144); the reaction was exothermic. Precipitated sodium bromide was removed and the alcoholic solution fractionated. After being washed in ethereal solution with water, the *product* (10·4 g.; 85%) distilled at 178°/25 mm. as a colourless evil-smelling liquid (Found: C, 66·3; H, 5·5. C_9H_9NS requires C, 66·3; H, 5·5%).

p-*Amidinobenzyl Methyl Sulphide Hydrochloride* (IX).—A solution of the above nitrile (10 g.) in chloroform (30 c.c.) and absolute alcohol (13 c.c.) was saturated with dry hydrogen chloride at 0° and kept in the ice-chest for 7 days. Solvent and excess of hydrogen chloride were removed in a vacuum at room temperature and the resulting imino-ether hydrochloride was set aside at 37° with 10% alcoholic ammonia solution (100 c.c.) for several days. The gum, obtained on removal of the solvent by distillation, was dissolved in water, and the solution, brought to about pH 6 with hydrochloric acid, was treated with " norite " and evaporated to dryness. The *product* (10·8 g.) separated from a small volume of water in colourless prisms, m. p. 80°, and, after being dried at 100°/vac., 139—140° (Found: C, 46·1; H, 6·4; loss at 100°/vac., 7·7. $C_9H_{12}N_2S,HCl,H_2O$ requires C, 46·1; H, 6·4; H_2O, 7·8%).

The *acetate*, obtained by double decomposition in concentrated aqueous solution, crystallised from water in radiating clusters of flattened needles, m. p. 243—244° (Found: C, 55·0; H, 6·9. $C_9H_{12}N_2S,C_2H_4O_2$ requires C, 55·0; H, 6·7%).

p-(*Aminomethyl*)*benzyl Methyl Sulphide Hydrochloride* (X).—Aqueous potassium hydroxide (25 g. in 50 c.c.) was dropped into a boiling stirred suspension of chromous acetate (from 50 g. of potassium dichromate) in an alcoholic solution (150 c.c.) of *p*-cyanobenzyl methyl sulphide (7 g.), through which hydrogen was bubbled continuously. Heating and stirring were maintained for 30 minutes after the addition of the alkali, and the mixture was thereafter acidified and steam-distilled. An ethereal extract of this distillate afforded an oil (4·7 g.), yielding unchanged starting material (3·6 g.), b. p. 170°/20 mm., on fractionation. The residual mixture from the steam-distillation was made strongly alkaline and again steam-distilled, the volatile base being neutralised with N-hydrochloric acid. The *product*, freed from some ammonium chloride (0·45 g.) by filtration of a solution in absolute alcohol, separated from *iso*propyl alcohol in colourless, irregular plates (0·4 g.), m. p. 250—254° (Found: C, 53·2; H, 6·7. $C_9H_{13}NS,HCl$ requires C, 53·1; H, 6·9%).

p-*Cyanobenzyl Methyl Sulphone* (V).—A solution of *p*-cyanobenzyl methyl sulphide (5 g.) in glacial acetic acid (10 c.c.) was cautiously treated with 30% hydrogen peroxide (8 c.c.). In the course of a week at room temperature a crystalline solid separated; water was then added and the substance collected. The *product* separated from alcohol in colourless needles (6 g.), m. p. 166° (Found: C, 55·7; H, 4·6; N, 7·5. $C_9H_9O_2NS$ requires C, 55·4; H, 4·6; N, 7·2%).

p-*Amidinobenzyl Methyl Sulphone Hydrochloride* (VII).—The preceding cyano-sulphone (13 g.) was converted into the imino-ether hydrochloride in the usual way. The latter, freed from solvents and excess of hydrogen chloride in a vacuum at room temperature, was incubated with 10% alcoholic ammonia (120 c.c.) at 37° for 4 days, during which time a heavy crystalline solid separated. The substance was separated and combined with the residue left on evaporation of the solvent. A solution of the combined solids in water was brought to pH 6 with hydrochloric acid, freed from a small amount (0·8 g.) of unchanged nitrile, decolourised with " norite ", and evaporated to dryness. The *product* crystallised from a small volume of water in colourless prisms (13 g.), m. p. 284° (Found: C, 43·6; H, 5·5; N, 11·4. $C_9H_{12}O_2N_2S,HCl$ requires C, 43·5; H, 5·2; N, 11·2%).

The *benzoate*, prepared by double decomposition in aqueous solution, crystallised from water in elongated rectangular plates, m. p. 244° (Found: C, 57·3; H, 5·4; N, 8·1. $C_9H_{12}O_2N_2S,C_7H_6O_2$ requires C, 57·5; H, 5·4; N, 8·4%).

p-(*Aminomethyl*)*benzyl Methyl Sulphone Hydrochloride* (VIII).—*p*-Cyanobenzyl methyl sulphone (12 g.) was hydrogenated in 10% alcoholic ammonia (130 c.c.) in the presence of Raney nickel (4 g.) at an initial pressure of 26 atm., the temperature being allowed to rise to 60° during the reaction by the application of external heat. Catalyst was removed by filtration and solvent by distillation. The resulting solid was neutralised with N-hydrochloric acid (required, 58 c.c.; calc., 60 c.c.), and the solution, decolourised with " norite," was evaporated to dryness. The *product* separated from 90% alcohol in tiny, irregular, colourless plates (11·5 g.), m. p. 272—274° (Found: C, 45·9; H, 6·2; N, 5·8. $C_9H_{13}O_2NS,HCl$ requires C, 46·0; H, 6·0; N, 6·0%).

p-*Methylsulphonylphenylacetamide* (XI).—*p*-Methylsulphonylacetophenone (3 g.) (Fuller, Tonkin, and Walker, *loc. cit.*) in dioxan (9 c.c.) was heated for 8 hours at 160° in a sealed tube with sulphur (1 g.) in ammonium sulphide solution (10 g.), obtained by saturating aqueous ammonia (*d* 0·88) with hydrogen sulphide. On cooling, a thick oily layer separated and partly solidified on treatment with water. The crude solid (2·2 g.) was digested with ammonium sulphide solution (100 c.c.) on the water-bath for ½ hour, cooled, and collected. On crystallisation from water (" norite "), the *product* separated in colourless needles (1 g.), m. p. 200° (Found: C, 50·6; H, 5·1; N, 6·6. $C_9H_{11}O_3NS$ requires C, 50·7; H, 5·1; N, 6·6%).

A number of modifications of the foregoing experiment were examined, but no significant improvement in yield resulted.

p-*Methylsulphonylphenylthioacetmorpholide* (XII).—*p*-Methylsulphonylacetophenone (5 g.) was heated in an oil-bath at 130—150° for 8 hours with morpholine (2·3 g.) and sulphur (0·8 g.). The thick oil so obtained rapidly crystallised on treatment with ethyl acetate, affording material (4 g.), m. p. 142—143°, sufficiently pure for subsequent use directly. Evaporation of the ethyl acetate and crystallisation of the residue from methyl alcohol (" norite ") gave a further quantity (1 g.) of the same material. The pure *compound* separated from methyl alcohol in yellow rectangular prisms, m. p. 143·5° (Found: C, 52·3; H, 6·0; N, 4·8. $C_{13}H_{17}O_3NS_2$ requires C, 52·1; H, 5·7; N, 4·7%).

p-*Methylsulphonylphenylacetic Acid*.—The preceding compound (16 g.) was refluxed for 13 hours with 2N-potassium hydroxide (190 c.c.), and the solution was then cooled, filtered from a small amount of sulphur, and acidified. The *product* (9·2 g.) separated from water in clusters of colourless needles, m. p. 137° (Found: C, 50·7; H, 5·0. $C_9H_{10}O_4S$ requires C, 50·5; H, 4·7%).

The *ethyl* ester (9·5 g.), obtained by refluxing the acid (9·1 g.) with absolute alcohol (25 c.c.) and concentrated sulphuric acid (1·8 c.c.) for 3 hours, separated from benzene–ligroin in colourless felted needles, m. p. 79° (Found: C, 54·7; H, 5·8. $C_{11}H_{14}O_4S$ requires C, 54·5; H, 5·8%). The amide (XI), m. p. 200°, was obtained in good yield by treating this ester with concentrated aqueous ammonia.

p-*Methylsulphonylphenylacetonitrile*.—*p*-Methylsulphonylphenylacetamide (6·4 g.) was refluxed with phosphoryl chloride (6·3 c.c.) in chloroform (20 c.c.) for 20 minutes. Excess of oxychloride and solvent

were then removed and the residue was treated with ice and water. The *product* separated from water in colourless, rectangular plates (5 g.), m. p. 124° (Found : C, 55·7; H, 4·6; N, 7·0. $C_9H_9O_2NS$ requires C, 55·4; H, 4·6; N, 7·2%). The mother-liquors on concentration afforded a mixture of nitrile and amide.

p-*Methylsulphonylphenylacetamidine Hydrochloride* (XIII).—The imino-ether hydrochloride prepared from the above nitrile (4·4 g.) was incubated with 10% alcoholic ammonia in the manner described above. The alcoholic ammonia solution was filtered from a small amount of ammonium chloride and evaporated to dryness, affording a sticky gum. The gum was dissolved in water and the solution was brought to pH 6 with hydrochloric acid, decolourised with " norite," and evaporated to dryness. The last traces of water were removed by repeated evaporation to dryness with absolute alcohol in a vacuum on the water-bath, affording a stiff glass (4·2 g.). The *product* eventually crystallised from methyl alcohol–ethyl acetate, or from *n*-propyl alcohol, in fine needles (2·4 g.), m. p. 175—177°, softening at 170° (Found : C, 43·9; H, 5·1. $C_9H_{12}O_2N_2S,HCl$ requires C, 43·5; H, 5·2%). Unlike the amidines described above, this one did not yield a sparingly soluble benzoate, and attempts to prepare other salts less soluble than the hydrochloride, such as the acetate or the nitrate, were unsuccessful.

β-p-*Methylsulphonylphenylpropionamide*.—β-*p*-Methylsulphonylphenylpropionyl chloride (from 15 g. of the acid) (Fuller, Tonkin, and Walker, *loc. cit.*) was dissolved in acetone and slowly added to aqueous ammonia (100 c.c.; *d* 0·88). The *product* (13·2 g.) separated from water in rectangular plates, m. p. 182° (Found : C, 52·7; H, 5·5. $C_{10}H_{13}O_3NS$ requires C, 52·9; H, 5·7%).

β-p-*Methylsulphonylphenylpropionitrile* (XV).—The above amide (11·7 g.) was dehydrated with phosphoryl chloride in chloroform in the manner described for the lower homologue (above). The *nitrile* (9 g.) separated from aqueous alcohol in rectangular prisms, m. p. 96° (Found : C, 57·0; H, 5·0; N, 6·8. $C_{10}H_{11}O_2NS$ requires C, 57·4; H, 5·3; N, 6·7%).

β-p-*Methylsulphonylphenylpropionamidine Hydrochloride* (XVI).—The above nitrile (4·25 g.) was converted into the amidine by way of the imino-ether hydrochloride. The crude product separated during the digestion with ammonia as a heavy crystalline precipitate. The *compound* crystallised from spirit in irregular, colourless plates (4·4 g.), m. p. 213°, softening at 209° (Found : C, 45·8; H, 5·7. $C_{10}H_{14}O_2N_2S,HCl$ requires C, 45·7; H, 5·7%).

The *benzoate*, obtained by double decomposition, separated from water in rectangular plates, m. p. 219°, softening at 213° (Found : C, 58·5; H, 5·5. $C_{10}H_{14}O_2N_2S,C_7H_6O_2$ requires C, 58·6; H, 5·7%).

γ-p-*Methylsulphonylphenyl*-n-*propylamine Hydrochloride* (XVII).—β-*p*-Methylsulphonylphenylpropionitrile (4·5 g.) was hydrogenated in saturated alcoholic ammonia (50 c.c.) at 50 atm. in the presence of Raney nickel (1 g.). The temperature was raised to about 70° in the course of 2 hours, and the autoclave was then allowed to cool. The catalyst was collected, and the solvent was evaporated under reduced pressure. The residue was taken up in water and neutralised with N-hydrochloric acid (required, approx. 12 c.c.; calc. 21·5 c.c.). A small volume of alcohol was added to give a homogeneous solution, which was then decolourised with " norite " and evaporated to small bulk. On cooling, unchanged nitrile (2·4 g.) separated and was collected. On further evaporation to dryness and crystallisation from absolute alcohol, the *product* separated in colourless square plates (1·3 g.), m. p. 214—215° (Found : C, 48·3; H, 6·2. $C_{10}H_{15}O_2NS,HCl$ requires C, 48·1; H, 6·4%).

Diazomethyl β-p-*Methylsulphonylphenylethyl Ketone*.—β-*p*-Methylsulphonylphenylpropionyl chloride (from 10·5 g. of acid) was dissolved in a mixture of ether (50 c.c.) and chloroform (50 c.c.), and added to a dried ethereal solution of diazomethane (from 20·5 g. of nitrosomethylurea). After standing overnight, the product, which had separated, was collected (8·5 g.; m. p. 95—97°); it was sufficiently pure for further use directly. The pure *compound* separated from benzene–ligroin in pale yellow needles, m. p. 97° (Found : C, 52·8; H, 4·7. $C_{11}H_{12}O_3N_2S$ requires C, 52·4; H, 4·8%).

γ-p-*Methylsulphonylphenyl*-n-*butyramide*.—The preceding diazo-ketone (9·2 g.) in dioxan (46 c.c.) was treated with aqueous ammonia (60 c.c. of ammonia solution, *d* 0·88; 9 c.c. of water) and 10% aqueous silver nitrate (14 c.c.) at 60—70°. After a few minutes a brisk evolution of nitrogen took place, and after this slackened the reaction was completed at the boiling point for a short time. The solution was filtered hot, diluted with water, and decolourised with " norite." The *product*, which separated on concentration to small bulk, crystallised from water in colourless fern-like needles (6·7 g.), m. p. 134° (Found : C, 54·9; H, 6·0. $C_{11}H_{13}O_3NS$ requires C, 54·8; H, 6·2%).

As a by-product in one experiment *chloromethyl* β-p-*methylsulphonylphenylethyl ketone* was isolated. This separated from methyl alcohol in colourless prisms, m. p. 114° (Found : C, 51·1; H, 5·4. $C_{11}H_{13}O_3SCl$ requires C, 50·7; H, 5·0%). On condensation with thiourea (0·27 g.) in alcohol (7 c.c.) on the water-bath for 2 hours, this compound (0·9 g.) afforded 4-β-p-*methylsulphonylphenylethyl*-2-*aminothiazole hydrochloride* (1·1 g.), which crystallised on cooling. Recrystallisation from methyl alcohol afforded colourless plates, m. p. 219—220° (Found : N, 8·4. $C_{12}H_{14}O_2N_2S_2,HCl$ requires N, 8·8%).

γ-p-*Methylsulphonylphenyl*-n-*butyronitrile*.—γ-*p*-Methylsulphonylphenyl-*n*-butyramide (8·2 g.) was dehydrated with phosphoryl chloride (8 g.) in chloroform (40 c.c.). On working up the reaction mixture a solid was obtained which was taken up in chloroform. The chloroform solution was dried and evaporated. The residue, on distillation in a high vacuum, crystallised in the receiver in the form of tiny rectangular plates (6·7 g.), m. p. 66°. The *product* had m. p. 70° after crystallisation from carbon tetrachloride, the only suitable solvent (Found : C, 58·8; H, 6·1; N, 6·5. $C_{11}H_{13}O_2NS$ requires C, 59·2; H, 5·8; N, 6·3%).

γ-p-*Methylsulphonylphenyl*-n-*butyramidine Hydrochloride* (XVIII).—The preceding nitrile (3 g.) was converted into the amidine by way of the imino-ether hydrochloride. A clear solution resulted at the end of the digestion with alcoholic ammonia. The residue obtained on evaporation to dryness was dissolved in water, brought to pH 6, decolourised with " norite," and recovered by evaporation to dryness. The last traces of water were removed by evaporation under reduced pressure with absolute alcohol. A concentrated solution in absolute alcohol was filtered from traces of ammonium chloride, and the alcohol was again removed. On crystallisation from methyl alcohol–ethyl acetate, the *product* separated in colourless rectangular prisms (3 g.), m. p. 141—142° (Found : C, 48·0; H, 6·3. $C_{11}H_{16}O_2N_2S,HCl$ requires C, 47·7; H, 6·2%).

The *benzoate* crystallised from water in elongated, rectangular plates, m. p. 185° (Found: C, 59·7; H, 6·3. $C_{11}H_{16}O_2N_2S,C_7H_6O_2$ requires C, 59·7; H, 6·1%).

δ-p-*Methylsulphonylphenyl*-n-*butylamine Hydrochloride* (XIX).—γ-*p*-Methylsulphonylphenyl-*n*-butyronitrile (3 g.) was reduced in alcoholic ammonia solution (170 c.c., saturated) in the presence of Raney nickel (1 g.) in hydrogen at a pressure of 57 atm., the temperature being raised to 50° during the reaction. After 4 hours the reaction mixture was worked up. Unchanged nitrile (1·6 g.) was recovered, and the *product* crystallised from methyl alcohol–ether in colourless, irregular plates (1·2 g.), m. p. 158—159° (Found: C, 50·0; H, 6·8; N, 5·6. $C_{11}H_{17}O_2NS,HCl$ requires C, 50·1; H, 6·8; N, 5·3%).

Note on the Physical Properties of the Above Amidine Salts.—The amidine hydrochlorides (I), (XIII), (XVI), and (XVIII) presented the noteworthy feature of showing alternations in the melting points and solubilities as the homologous series was ascended. The melting points followed the sequence: 294°, 175—177°, 213°, and 141—142°. Similarly the solubilities in water were alternately moderate and excessively high.

Ethyl p-*Methylsulphonylbenzoylacetate* (XX).—*p*-Methylsulphonylbenzoyl chloride (from 20 g. of acid; 1 mol.) in benzene (120 c.c.) was added to ethyl sodioacetoacetate (from 4·6 g. of sodium and 26 g. of ethyl acetoacetate; 2 mols.) in benzene (300 c.c.). The mixture was refluxed for 4 hours and then allowed to stand overnight. After addition of excess of dilute sulphuric acid a small amount of *p*-methylsulphonylbenzoic acid (2·9 g.) was recovered by filtration. The benzene layer was evaporated and the residue treated with methyl alcohol (250 c.c.) containing sodium methoxide (from 5 g. of sodium). After 5½ hours at room temperature the solution was poured into a mixture of ice and dilute sulphuric acid, and the precipitated oil was extracted with ether. The residue, obtained on evaporation of the dried ether extract, was fractionally crystallised from methyl alcohol. The first fraction (2·8 g.), m. p. 118—119°, was shown to be methyl *p*-methylsulphonylbenzoate by comparison with an authentic specimen (below). The second fraction (5·8 g.), m. p. 80—90°, after crystallisation from benzene and again from methyl alcohol, afforded the pure *product* (XX), m. p. 86—88°, in the form of colourless rectangular plates (Found: C, 53·7; H, 5·1. $C_{12}H_{14}O_5S$ requires C, 53·3; H, 5·2%).

The substance gave a typical blood-red ferric reaction in alcoholic solution and it was further characterised as the *phenylpyrazolone*. The keto-ester (0·24 g.) and phenylhydrazine (0·1 c.c.) were heated on the water-bath for 3 hours. The thick gum solidified on trituration with ether and the solid (0·1 g.), after crystallisation from dilute acetic acid, had m. p. 215° (Found: N, 9·3. $C_{16}H_{14}O_3N_2S$ requires N, 9·0%).

Methyl p-*Methylsulphonylbenzoate.*—*p*-Methylsulphonylbenzoic acid (2 g.) was esterified with methyl alcohol (10 c.c.) in the presence of concentrated sulphuric acid (0·5 c.c.) on the water-bath for 2 hours. The *product*, obtained on diluting the reaction mixture with water, separated from methyl alcohol in elongated, rectangular colourless plates, m. p. 118—119° (Found: C, 50·1; H, 4·8. $C_9H_{10}O_4S$ requires C, 50·5; H, 4·7%).

2-*Phenyl*-4-p-*methylsulphonylbenzylidene*-5-*oxazolone.*—*p*-Methylsulphonylbenzaldehyde (9·2 g.) (Fuller, Tonkin, and Walker, *loc. cit.*), hippuric acid (9·6 g.), and sodium acetate (4 g.) were intimately mixed. Acetic anhydride (14 c.c.) was then added and the paste was heated until fusion occurred and then left on the water-bath for 2 hours. The mixture was then cooled, treated with absolute alcohol (20 c.c.), and left in the ice-chest overnight. The product (13 g.) was then collected, and washed with two successive portions of boiling water (5 c.c.) and with a little cold water. The m. p. was 182—184° and the material was sufficiently pure for further use directly. The pure *compound* separated from acetic acid in yellow needles, m. p. 184—186° (Found: C, 62·7; H, 3·8; N, 4·2. $C_{17}H_{13}O_4NS$ requires C, 62·4; H, 4·0; N, 4·3%).

β-p-*Methylsulphonylphenylalanine* (XXI).—To the crude azlactone (11·8 g.), mixed with red phosphorus (7 g.) and acetic anhydride (44 c.c.), hydriodic acid (41 c.c.; *d* 1·7) was added slowly, with constant shaking, during 30 minutes. The solution was then refluxed for 3 hours, cooled, freed from phosphorus by filtration, and evaporated to dryness. The residue was taken up in water (30 c.c.), again evaporated to dryness, and again redissolved in water (100 c.c.). The aqueous solution was thrice extracted with ether, treated with " norite " after removal of dissolved ether, and then heated to the boiling point. On bringing the pH to about 4 and cooling, the product (7·5 g.) separated. It was recrystallised with difficulty from water when the pure *compound* separated as a micro-crystalline powder, m. p. 250—256° (Found: C, 49·8; H, 5·5; N, 5·6. $C_{10}H_{13}O_4NS$ requires C, 49·4; H, 5·3; N, 5·8%).

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302. *Chemotherapeutic Agents of the Sulphone Type. Part IV.*

By H. S. Forrest and James Walker.

4-γ-Diethylaminopropylamino-2-p-methylsulphonylphenyl-6-methylpyrimidine dihydrochloride has been synthesised from *p*-methylsulphonylbenzamidine hydrochloride, and a number of thiazoles have been prepared from α-halogenated ketones and p-*methylsulphonylbenzthioamide*. None of the products possessed antimalarial activity.

p-Methylsulphonylbenzamidine hydrochloride (I) has been shown to have definite prophylactic activity and a slight therapeutic action in *P. gallinaceum* infections in chicks (Fuller, Tonkin, and Walker, *J.*, 1945, 633). It was also shown that prophylactic activity was retained in low degree by the amidoxime corresponding to (I) and by the *NN*-dimethyl derivative of (I), but lost, for example, on introducing a γ-diethylaminopropyl substituent into the amidine

group of (I). A few exploratory experiments have been carried out in attempts to develop antimalarial compounds from (I) and from its precursor, *p*-methylsulphonylbenzonitrile, by way of p-*methylsulphonylbenzthioamide* (II), and these are now reported.

Condensation of (I) with ethyl acetoacetate in the presence of enough alkali to liberate the free base gave 4-*hydroxy*-2-p-*methylsulphonylphenyl*-6-*methylpyrimidine*, which afforded 4-*chloro*-2-p-*methylsulphonylphenyl*-6-*methylpyrimidine* (III) on treatment with phosphoryl chloride. Condensation of (III) with γ-diethylaminopropylamine then gave 4-*γ-diethylaminopropylamino*-2-p-*methylsulphonylphenyl*-6-*methylpyrimidine dihydrochloride* (IV) in good yield. Addition of the elements of hydrogen sulphide to *p*-methylsulphonylbenzonitrile afforded *p*-methylsulphonylbenzthioamide (II) quantitatively. With phenacyl bromide and ethyl α-chloroacetoacetate, (II) afforded 4-*phenyl*-2-p-*methylsulphonylphenylthiazole* (V) and *ethyl* 2-p-*methylsulphonylphenyl*-4-*methylthiazole*-5-*carboxylate* (VI) respectively, while 1 : 12-dichloro-2 : 11-diketododecane and 1 : 14-dichloro-2 : 13-diketotetradecane yielded, on condensation with (II), 1 : 8-*bis*-2′-p-*methylsulphonylphenyl*-4′-*thiazolyl*-n-*octane* (VII) and 1 : 10-*bis*-2′-p-*methylsulphonylphenyl*-4′-*thiazolyl*-n-*decane* (VIII) respectively.

None of the above substances (IV), (V), (VI), and (VII) showed any antimalarial activity when tested in *P. gallinaceum* infections in chicks. Curd and Rose (*J.*, 1946, 343) have examined a short series of 4-β-diethylaminoethylamino-2-aryl-6-methylpyrimidine dihydrochlorides and again no antimalarial activity was observable.

Experimental.

4-*Hydroxy*-2-p-*methylsulphonylphenyl*-6-*methylpyrimidine*.—A mixture of *p*-methylsulphonylbenzamidine hydrochloride (20 g.) and ethyl acetoacetate (13 g.) was warmed to about 40° and treated with a solution of sodium hydroxide (4 g.) in water (40 c.c.). The clear solution which resulted soon began to deposit crystals. After 2 days at 37° the product was collected, dissolved in excess of dilute aqueous sodium hydroxide, and reprecipitated by acetic acid after filtration. On recrystallisation from alcohol, the *compound* separated in colourless needles (22 g.), m. p. 260° (Found : C, 54·5; H, 4·5; N, 10·4. $C_{12}H_{12}O_3N_2S$ requires C, 54·5; H, 4·5; N, 10·6%).

4-*Chloro*-2-p-*methylsulphonylphenyl*-6-*methylpyrimidine* (III).—The above hydroxy-compound (21 g.) was refluxed for 30 minutes with phosphoryl chloride (50 c.c.). Excess of reagent was removed under reduced pressure and the residue was treated with ice and left overnight. The precipitate was collected, and, on crystallisation from absolute alcohol, the *product* separated in pale yellow needles (17 g.), m. p. 139° (Found : C, 51·2; H, 3·9; N, 9·8. $C_{12}H_{11}O_2N_2ClS$ requires C, 51·0; H, 3·9; N, 9·9%).

4-*γ-Diethylaminopropylamino*-2-p-*methylsulphonylphenyl*-6-*methylpyrimidine Dihydrochloride* (IV).—The preceding chloro-compound (10 g.) was heated at 115—125° for 6 hours with γ-diethylaminopropylamine (6 g.; 1·25 mols.). The mixture was then cooled and dissolved in 2N-hydrochloric acid, and the solution was filtered. The solution was made alkaline with sodium hydroxide and the base was taken up in ether. The ethereal solution, in turn, was extracted with dilute acetic acid. The acid extract was then basified and the mixture was again extracted with ether. After being dried (K_2CO_3), the ether extract, on evaporation, afforded a thick brown syrup which was dissolved in benzene and treated with dry hydrogen chloride; a semi-solid precipitate then formed. The benzene was decanted and the residue dissolved in hot alcohol and treated with an equal volume of ethyl acetate. On cooling, the *product* separated in clumps of colourless needles (12 g.), m. p. 247° (Found : C, 50·6; H, 6·9. $C_{19}H_{28}O_2N_4S,2HCl$ requires C, 50·9; H, 6·7%).

p-*Methylsulphonylbenzthioamide* (II).—*p*-Methylsulphonylbenzonitrile (20 g.), alcoholic ammonia (30 c.c.; 10%), and alcohol (33 c.c.) were placed in a pressure bottle and the mixture was then saturated with hydrogen sulphide and heated in a boiling water-bath for 1½ hours. The solvent was removed under reduced pressure and the residue was washed with ammonium sulphide solution. The *product* separated from a large volume of water in pale yellow needles (21 g.), m. p. 205—206°, varying somewhat with the rate of heating (Found : C, 44·9; H, 4·1; N, 6·7. $C_8H_9O_2NS_2$ requires C, 44·6; H, 4·2; N, 6·5%).

4-*Phenyl*-2-p-*methylsulphonylphenylthiazole* (V).—A solution of the above thioamide (3 g.) and phenacyl bromide (3 g.) in alcohol (100 c.c.) was refluxed for 2 hours; the thioamide dissolved completely and then colourless crystals separated. The solution was cooled, and the product (4·2 g.) collected. The *compound* crystallised from alcohol in colourless hexagonal plates, m. p. 186° (Found : C, 60·5; H, 4·3; N, 4·3. $C_{16}H_{13}O_2NS_2$ requires C, 61·0; H, 4·2; N, 4·4%).

Ethyl 2-p-*Methylsulphonylphenyl*-4-*methylthiazole*-5-*carboxylate* (VI).—A mixture of *p*-methylsulphonylbenzthioamide (5 g.), ethyl α-chloroacetoacetate (5 g.), and spirit (100 c.c.) was refluxed for 2 hours. The process was repeated after addition of a further quantity (3 g.) of chloro-ester. The crystalline *product*, obtained on cooling, was collected and recrystallised from alcohol; colourless rectangular plates (6·5 g.) separated, m. p. 151° (Found : C, 51·5; H, 4·7; N, 4·0. $C_{14}H_{15}O_4NS_2$ requires C, 51·7; H, 4·6; N, 4·3%).

1 : 8-*Bis*-2′-p-*methylsulphonylphenyl*-4′-*thiazolyl*-n-*octane* (VII).—A mixture of 1 : 12-dichloro-2 : 11-diketododecane (4·1 g.), *p*-methylsulphonylbenzthioamide (6·7 g.), and absolute alcohol (100 c.c.) was refluxed for 4 hours, and, after cooling, the product (8·8 g.; m. p. 125—130°) was collected. Extraction with hot alcohol left a residue (4·2 g.; m. p. 152°), and concentration of the extract afforded more of the same material (3·3 g.; m. p. 148—150°) and finally unchanged thioamide (0·5 g.). The *product* separated from glacial acetic acid in tiny needles (6·3 g.), m. p. 152·5° (Found : C, 57·2; H, 5·4. $C_{28}H_{32}O_4N_2S_4$ requires C, 57·2; H, 5·4%).

1 : 10-*Bis*-2′-p-*methylsulphonylphenyl*-4′-*thiazolyl*-n-*decane* (VIII).—In the same manner, 1 : 14-dichloro-2 : 13-diketotetradecane (2·7 g.) and *p*-methylsulphonylbenzthioamide (4 g.) afforded a crude product (5·1 g.; m. p. 105—115°), similarly purified by extraction with alcohol. The *compound* (3 g.) separated from glacial acetic acid in small needles (2·2 g.), m. p. 150° (Found: C, 58·8; H, 5·9. $C_{30}H_{36}O_4N_2S_4$ requires C, 58·4; H, 5·8%).

The authors are greatly indebted to Miss I. M. Tonkin, B.Sc., for undertaking the antimalarial tests.

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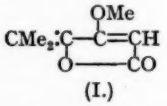
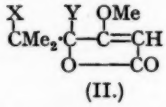
303. *Compounds related to Penicillic Acid. Part III. Synthesis of Penicillic Acid.*

By R. A. RAPHAEL.

The course of several attempted syntheses of penicillic acid (III; Y = OH) is detailed. The successful synthesis is described.

As a starting point for the synthesis of penicillic acid the readily obtainable lactone (I) (Raphael, *J.*, 1947, 805) was the logical choice. It would be reasonable to expect that addition to the exocyclic double bond, giving an adduct of type (II), followed by elimination of HX, would lead to a compound of type (III), a simple derivative of penicillic acid (III; Y = OH).


(I.)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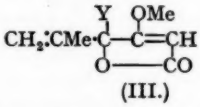
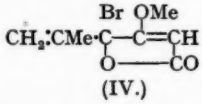

(III.)

The first addendum to be tried was bromine; this readily reacted with a glacial acetic acid solution of the lactone (I) with concomitant evolution of hydrogen bromide. The crystalline product obtained analysed for a *monobromo-lactone* for which the three structures (IV)—(VI) were possible. The first could arise by addition and elimination as suggested above, (V) could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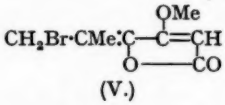
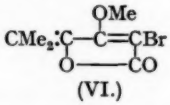
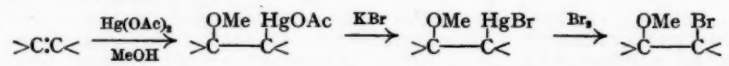

(V.)


(VI.)

be formed by the same process followed by an anionotropic rearrangement, and a simple substitution could yield (VI). The ultra-violet light absorption of the compound showed a maximum at 2820 A., thus indicating the presence of a triply conjugated system; this at once eliminated (IV) which would be expected to exhibit maximal absorption at about 2200 A. Examination of the chemical properties of the compound revealed that the bromine atom was very firmly bound; for example, refluxing with potassium acetate in glacial acetic acid for 24 hours yielded only unchanged material, and boiling with alcoholic potash had to be continued for 15 minutes before formation of potassium bromide started. This property strongly favoured the constitution (VI), and confirmation was provided by ozonolysis, whereby acetone was obtained in 57% yield as its 2 : 4-dinitrophenylhydrazone. A bromo-lactone of this structure was of course unsuitable for further relevant synthetic use.

The next addition to be attempted on lactone (I) involved the use of the benzene-soluble iodine–silver benzoate complex (*inter alii*, Prevost and Wiemann, *Compt. rend.*, 1937, **204**, 989; Hershberg, *Helv. Chim. Acta*, 1934, **17**, 351) in the hope of obtaining (II; X = Y = OBz). The only product isolated, however, was the *monoiodo-lactone* corresponding to (VI) in structure.

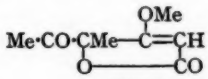
Recourse was then had to the methoxymercuration reaction (*Org. Synth.*, Vol. 20, pp. 81, 101; Romeyn and Wright, *J. Amer. Chem. Soc.*, 1947, **69**, 697). This consists in reaction of an ethylenic compound with a solution of mercuric acetate in methanol at room temperature and treating the resulting adduct as follows:

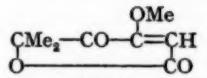


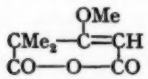
As is seen, the net effect is that of adding the elements of methyl hypobromite across the double bond. Application of this reagent to lactone (I) might be expected to yield (II; X = Br,

Y = OMe), from which methyl penicillate should be obtainable by elimination of hydrogen bromide. Unfortunately, mercuration occurred in the ring, and treatment of the mercury complex with potassium bromide and bromine yielded the same monobromo-lactone as had been produced by direct bromination.

It was then found that the lactone (I) reacted smoothly with performic acid (Swern, Billen, Findlay, and Scanlon, *J. Amer. Chem. Soc.*, 1945, **67**, 1786) to yield the *dihydroxy*-compound (II; X = Y = OH). This constitution was confirmed by fission with periodic acid whereby acetone (estimated as its 2 : 4-dinitrophenylhydrazone) was furnished in 82% yield; further proof of the vicinal position of the hydroxyl groups was provided by the preparation of an iso*propylidene* derivative with acetone. It was to be expected that dehydration of the dihydroxy-compound would yield penicillic acid directly, but relatively mild dehydrating agents led to recovery of unchanged starting material (acetic anhydride gave the *diacetyl* compound) and vigorous methods produced a neutral *ketone* (2 : 4-*dinitrophenylhydrazone*) obviously derived from the dihydroxy-compound by means of a pinacolone rearrangement. There are three theoretical ways in which this could occur, yielding the products (VII)—(IX). Structure (IX)

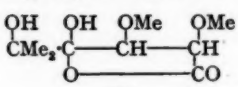

(VII.)

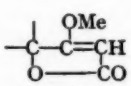

(VIII.)

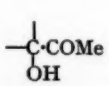

(IX.)

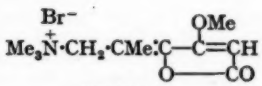
can be eliminated at once as it would not show ketonic properties. The discriminating factor between the other two was provided by the ultra-violet absorption properties of the compound, a maximum appearing at 2650 A.; the presence of a triply conjugated system as in (VIII) was thus indicated. A compound of structure (VII) would be expected to exhibit maximal absorption at about 2200 A. Confirmation was provided by alkaline hydrolysis of the ketone, whereby the dihydroxy-compound (II; X = Y = OH) was re-formed; this could only occur if the ketone possessed structure (VIII). The hydrolysis had to be carried out in an inert solvent, dioxan; when methanolic alkali was employed, addition of methanol occurred and the compound (X) was obtained.

It might be thought that the iodoform reaction would serve as a diagnostic test between (VII) and (VIII), and in fact the ketone yielded iodoform readily with sodium hypoiodite in the cold. Unfortunately, however, it has been found that all compounds in this field containing the ring system (XI) [*e.g.*, the lactone (I), the dihydroxy-compound (II; X = Y = OH), penicillic acid, and dihydropenicillic acid] gave a strong positive reaction, thus depriving the test of its usual validity. It is thought that under the conditions of the test the ring system (XI) is hydrolysed and decarboxylated to the methyl ketone (XII) which, of course, would react readily to give iodoform.


(X.)


(XI.)


(XII.)


(XIII.)

The successful synthesis was initiated by the observation that the lactone (I) reacted smoothly with *N*-bromosuccinimide in the presence of a trace of benzoyl peroxide (Schmid and Karrer, *Helv. Chim. Acta*, 1946, **29**, 573) to furnish a new *monobromo-lactone* possessing properties completely in harmony with structure (V). The compound obtained was a *cis–trans*-mixture, m. p. 74—95°, in which the bromine atom was very labile; it reacted exothermically with *cyclo*hexylamine (Ziegler, Späth, Schaaf, Schumann, and Winkelmann, *Annalen*, 1942, **551**, 80), and potassium acetate readily yielded the corresponding *acetyl* compound. Further verification was provided by ozonolysis, bromoacetone (estimated as 2 : 4-*dinitrophenylhydrazone*) being produced in 56% yield. This monobromo-lactone combined readily with trimethylamine in aqueous or ethereal solution to produce the *quaternary bromide* (XIII). On boiling an aqueous solution of (XIII) with excess of magnesium oxide, trimethylamine was evolved and, on acidification, penicillic acid was obtained in 87% yield.

The mechanism of the last step is formulated thus. The quaternary bromide (XIII) is first converted into the quaternary hydroxide, which then undergoes the rearrangement already encountered in this field (Raphael, *loc. cit.*); this has the net effect of adding the elements of water across the exocyclic double bond. Finally, occurrence of the familiar Hofmann reaction leads to penicillic acid.

The melting point of the synthetic acid was 86—87° undepressed on admixture with the

mould metabolic product; the acetyl derivative and the dibromide likewise gave no depression in melting point when mixed with the corresponding derivatives prepared from the natural product. The natural and the synthetic acid exhibited identical light absorption properties in the ultra-violet and infra-red.

Light absorptions determined in alcoholic solution.

	$\lambda_{max.}$, A.	$\varepsilon_{max.}$		$\lambda_{max.}$, A.	$\varepsilon_{max.}$
Lactone (I) [1]	2700	23,000	(II; X = Y = OH)	2240	13,500
Bromo-lactone (VI)	2820	18,500	(II; X = Y = OAc)	2300	12,500
Bromo-lactone (V)	2770	28,000	*iso*Propylidene compound of (II; X = Y = OH)	2370	11,500
Quaternary bromide (XIII)	2640	18,000	Penicillic acid (synthetic) ...	2255	10,500
Ketone (VIII)	2650	8,500	Penicillic acid (natural)	2255	10,500

[1] Raphael, *loc. cit.*

Experimental.

Bromination of Lactone (I).—The lactone (1·78 g.) was dissolved in glacial acetic acid (10 c.c.), and a solution of bromine (1·8 g.; 1·95 atoms) in glacial acetic acid (10 c.c.) dropped in slowly with shaking (the slight excess of lactone is essential for good yields). The solution rapidly decolourised and hydrogen bromide was evolved. The mixture was evaporated over solid potassium hydroxide in a vacuum desiccator, and the residual crystalline mass was extracted with boiling light petroleum (b. p. 80—100°). The extract on cooling deposited the *bromo-lactone* (VI) as a mass of needles (1·7 g.; 63%). Recrystallisation from the same solvent and from aqueous methanol gave needles, m. p. 121—122° (Found: C, 41·3; H, 3·9; Br, 33·7; OMe, 13·4. $C_8H_9O_3Br$ requires C, 41·25; H, 3·9; Br, 34·3; OMe, 13·3%).

Ozonolysis.—The bromo-lactone (VI) (180 mg.) was dissolved in purified acetic acid (10 c.c.), and ozonised oxygen passed in until absorption ceased. The resulting solution was poured into water (10 c.c.) containing zinc dust (5 g.), and the mixture subjected to steam-distillation. The first four 5-c.c. portions of distillate were treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate and allowed to stand overnight. The yellow precipitate was filtered off and dried on porous tile (105 mg.; 57%); crystallisation from alcohol gave yellow needles, m. p. 125°, undepressed on admixture with acetone 2 : 4-dinitrophenylhydrazone.

Action of Iodine–Silver Benzoate Complex on Lactone (I).—The lactone (1·03 g.), iodine (1·7 g.), and silver benzoate (3·05 g.) were heated under reflux overnight in dry benzene (150 c.c.). The solution was filtered, and the benzene evaporated. The solid residue was crystallised twice from alcohol and finally from light petroleum (b. p. 100—120°), forming needles, m. p. 154—156° (560 mg.; 31%). The analysis corresponded to that of a *monoiodo-lactone* (Found: C, 34·6; 34·6; H, 3·6, 3·2; OMe, 11·25. $C_8H_9O_3I$ requires C, 34·3; H, 3·25; OMe, 11·05%).

Action of Methanolic Mercuric Acetate on Lactone (I).—A solution of mercuric acetate (2·1 g.) and the lactone (1 g.) in methanol (10 c.c.) was kept for 3 days at room temperature. A solution of potassium bromide (1 g.) in water (5 c.c.) was added rapidly with stirring. The crystalline precipitate formed was filtered off, washed with water, and dried on porous tile in a vacuum. The thoroughly dry solid was dissolved in dry chloroform, and bromine in chloroform (10%) added at 0° until a faint permanent colour persisted. The mercuric bromide was filtered off and the chloroform evaporated, leaving a viscous oil which soon solidified to a mass of needles (860 mg.; 57%). The product crystallised from light petroleum (b. p. 80—100°) in needles, m. p. 119—120°. The analysis corresponded to that of a monobromo-lactone and the compound gave no depression of m. p. on admixture with the monobromo-lactone (VI) described above (Found: C, 41·25; H, 3·65%).

Action of Performic Acid on Lactone (I).—The lactone (1·5 g.) was dissolved in anhydrous formic acid (5 c.c.), and hydrogen peroxide (100-vol.; 2 c.c.) added; a vigorous reaction set in after about 10 minutes. After two hours' standing the solution was steam-distilled to remove formic acid and hydrolyse any formic ester, and the distillate evaporated to dryness under reduced pressure. The residual viscous liquid slowly solidified. Crystallisation from ethyl acetate or nitroethane yielded colourless prisms of the *dihydroxy*-compound (II; X = Y = OH) (1·3 g.; 71%), m. p. 148—150° (Found: C, 51·2; H, 6·65; OMe, 16·3. $C_6H_{12}O_5$ requires C, 51·05; H, 6·45; OMe, 16·5%). The compound reacted as a monobasic acid and was very soluble in water and other hydroxylic solvents. The *diacetyl* derivative, prepared in the usual manner, crystallised from light petroleum (b. p. 80—100°) in prismatic needles, m. p. 107—108° (Found: C, 53·25; H, 5·95. $C_{12}H_{16}O_7$ requires C, 52·95; H, 5·95%). The iso*propylidene* derivative, prepared in 42% yield by refluxing an acetone solution of the compound (1 g.) with anhydrous potassium carbonate (2 g.), crystallised in long needles, m. p. 102—103°, from light petroleum (b. p. 60—80°) (Found: C, 57·6; H, 6·85; OMe, 13·5. $C_{11}H_{16}O_5$ requires C, 57·9; H, 7·05; OMe, 13·6%).

Periodic Acid Fission of Dihydroxy-compound (II; X = Y = OH).—The dihydroxy-compound (470 mg.) was dissolved in water (5 c.c.), and a solution of periodic acid (570 mg.) in water (5 c.c.) added in one portion with cooling. After 16 hours the solution was steam-distilled and the first 30 c.c. of distillate were treated with excess of aqueous 2 : 4-dinitrophenylhydrazine sulphate. The yellow precipitate was filtered off, washed with water and dried on a porous tile (595 mg.; 82%). Crystallisation from alcohol gave needles, m. p. 126°, undepressed on admixture with acetone 2 : 4-dinitrophenylhydrazone.

Attempted Dehydration of Dihydroxy-compound (II; X = Y = OH).—Use of relatively mild dehydrating agents (iodine, toluene-*p*-sulphonic acid, magnesium sulphate, thionyl chloride in pyridine) led only to the recovery of unchanged starting material. More drastic methods (hot anhydrous formic

acid, anhydrous oxalic acid, phosphoric oxide, potassium pyrosulphate) yielded the ketone (VIII). As the method involving the last-named reagent produced the best yield, it alone will be described.

The dihydroxy-compound (1 g.) was intimately mixed with powdered potassium pyrosulphate (2 g.) and heated to 180° for 1 hour in an oil-bath. The cooled reaction mixture was treated with water to dissolve the potassium salt; the residual flocculent solid was filtered off, washed with water, and dried on porous tile. Crystallisation from light petroleum (b. p. 80—100°) furnished needles of the *ketone* (VIII), m. p. 96—97° (470 mg.; 52%) (Found: C, 56·85; H, 5·9; OMe, 18·2. $C_8H_{10}O_4$ requires C, 56·45; H, 5·9; OMe, 18·25%). The 2 : 4-*dinitrophenylhydrazone*, prepared by treating the ketone with alcoholic 2 : 4-dinitrophenylhydrazine sulphate, crystallised from *iso*amyl alcohol in orange plates, m. p. 228—229° (Kofler block) (Found: N, 15·65. $C_{14}H_{14}O_7N_4$ requires N, 16·0%).

Hydrolysis.—(a) A solution of the ketone (VIII) (366 mg.) in dioxan (5 c.c.) was treated with N-sodium hydroxide (1 mol.) and kept for 24 hours. N-Sulphuric acid (1 mol.) was added, and the solution evaporated to dryness at room temperature under reduced pressure. The residual solid was extracted with boiling ethyl acetate; the extract was evaporated to 2 c.c. and allowed to crystallise. The crystalline solid obtained (210 mg.; 51%) had m. p. 148—149° undepressed on admixture with the dihydroxy-compound (II; X = Y = OH).

(b) A solution of the ketone (VIII) (245 mg.) in methanol (3 c.c.) was treated exactly as in (a). The ethyl acetate extract was evaporated to dryness and the residual crystalline solid crystallised from benzene in needles (185 mg.; 58%), m. p. 98—99° depressed to 70—78° on admixture with the starting material. The *compound* (X) was acidic and showed no selective absorption in the ultra-violet (Found: C, 49·3; H, 7·35. $C_9H_{14}O_6$ requires C, 49·1; H, 7·3%).

Action of N-*Bromosuccinimide on Lactone* (I).—The lactone (5·8 g.) was dissolved in carbon tetrachloride (30 c.c.), *N*-bromosuccinimide (6·7 g.) and benzoyl peroxide (50 mg.) were added, and the mixture refluxed until the formation of succinimide was complete (*ca.* 2 hours). The mixture was cooled in ice, filtered, and the filtrate evaporated under reduced pressure; the pale yellow, residual oil slowly solidified to a mass of needles. Crystallisation from light petroleum (b. p. 100—120°) furnished the *bromo-lactone* (V) as plates, m. p. 74—95° (8·1 g.; 91%) (Found: C, 41·4; H, 4·1. $C_8H_9O_3Br$ requires C, 41·25; H, 3·9%). The compound possessed a marked irritant effect on the skin and mucous membrane. Mixing the substance with an equal weight of *cyclo*hexylamine resulted in a spontaneous rise in temperature to 190°. The corresponding *acetyl* compound, prepared by treatment with potassium acetate in glacial acetic acid, crystallised from light petroleum (b. p. 60—80°) in needles, m. p. 72—84° (Found: C, 56·55; H, 5·75. $C_{10}H_{12}O_5$ requires C, 56·6; H, 5·7%).

Ozonolysis.—The bromo-lactone (V) (500 mg.) was dissolved in carbon tetrachloride (25 c.c.), and ozonised oxygen bubbled through until no more gummy ozonide was precipitated. The solvent was carefully evaporated under reduced pressure, water (5 c.c.) added, and the mixture steam-distilled until the distillate remained clear. The turbid, strongly lachrymatory distillate was treated with excess of aqueous-alcoholic 2 : 4-dinitrophenylhydrazine sulphate; after 24 hours the yellow precipitate (383 mg.; 56%) was filtered off, washed with water, and dried on porous tile. Crystallisation from alcohol gave yellow needles, m. p. 124—125°, undepressed on admixture with authentic *bromoacetone* 2 : 4-*dinitrophenylhydrazone* (Found: N, 17·7. $C_9H_9O_4N_4Br$ requires N, 17·6%), but depressed by 15° when mixed with acetone 2 : 4-dinitrophenylhydrazone.

Quaternary Bromide (XIII).—(a) The bromo-lactone (V) (5·8 g.) was warmed gently by steam with aqueous trimethylamine (33%; 15 c.c.) until the solution became homogeneous. Evaporation to dryness under reduced pressure yielded a crystalline solid. The mass was triturated with dry acetone, filtered off, and washed well with more acetone. The hygroscopic *quaternary bromide* (5·3 g.; 73%) crystallised in clumps of needles from nitroethane and in plates from alcohol–ether; it had m. p. 145—148° (decomp.) (Found: C, 42·9; H, 6·4; N, 4·45; Br, 26·8. $C_{11}H_{18}O_5NBr$ requires C, 45·2; H, 6·2; N, 4·8; Br, 27·4%).

(b) The bromo-lactone (V) (2 g.) was dissolved in dry ether (20 c.c.), and a solution of trimethylamine (2 g.) in dry ether (20 c.c.) added. The mixture immediately became turbid and heat was evolved. After standing at room temperature for 24 hours the crystalline slurry was filtered off and the practically pure quaternary salt washed with dry ether (2·1 g.; 82%).

Penicillic Acid (III; Y = OH).—The quaternary bromide (XIII) (750 mg.) was dissolved in water (10 c.c.), magnesium oxide (750 mg.) added, and the suspension boiled in a stream of nitrogen until no more trimethylamine was evolved (90 mins.). The base was absorbed in a known volume of standard acid and back-titrated with standard alkali; the yield of trimethylamine was 92%. The reaction mixture was strongly acidified with 2N-sulphuric acid and extracted four times with ether. Drying ($MgSO_4$) and evaporation furnished an oil which partly solidified. Extraction with boiling *cyclo*hexane yielded penicillic acid (140 mg.; 32%), m. p. 86—87° after recrystallisation from the same solvent.

As it was noticed that most of the trimethylamine was evolved during the first ten minutes of boiling, the preparation was repeated employing this reaction time. Under these conditions the residual oil solidified completely and one crystallisation from *cyclo*hexane yielded pure penicillic acid (380 mg.; 87%) as needles, m. p. 86—87° (Found: C, 56·35, 56·15; H, 5·9, 5·7; OMe, 17·9, 17·5. Calc. for $C_8H_{10}O_4$: C, 56·45; H, 5·9; OMe, 18·25%). The synthetic acid gave no depression in m. p. when mixed with the natural product and exhibited the characteristic purple colouration on standing with concentrated ammonia (Birkinshaw, Oxford, and Raistrick, *Biochem. J.*, 1936, **30**, 394). Addition of bromine produced the dibromide, crystallising from benzene–light petroleum (b. p. 60—80°) in clumps of needles, m. p. 153—154°, undepressed on admixture with authentic penicillic acid dibromide (Birkinshaw, Oxford, and Raistrick, *loc. cit.*). Acetylation furnished the acetyl compound, which crystallised from light petroleum (b. p. 60—80°) in needles, m. p. 72—73° undepressed on admixture with authentic acetylpenicillic acid (Birkinshaw, Oxford, and Raistrick, *loc. cit.*).

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304. *The Replacement of the Diazonium by the Nitro-Group. Part IV. Decompositions involving Neutral Solutions of Diazonium Salts, with Facile Preparations of* o- *and* p-*Dinitrobenzene.*

By HERBERT H. HODGSON, FRED HEYWORTH, and EDWARD R. WARD.

The neutralised solutions of the diazonium salts from 13 amines have been caused to react with sodium nitrite in the presence of either cuprous oxide and copper sulphate or cupro-cupri sulphite. Yields of nitro-compounds have been obtained which compare favourably with those from methods earlier than the cobaltinitrite method (cf. Parts I and II), and are greatest when a nitro-group is already present in the initial amine. No catalysts are necessary with diazotised *o*- and *p*-nitroaniline, where the diazonium group is replaced by the nitro-group to the extent of 70 and 76% respectively.

A NEW method for the production of nitro-compounds, here described, is based on the reaction of a neutral aqueous solution of a diazonium salt with sodium nitrite in the presence of catalysts such as a mixture of cuprous oxide and cupric sulphate or the so-called cupro-cupri sulphite prepared by mixing equivalent aqueous solutions of cupric sulphate and sodium sulphite. The method is most useful when the reaction product is steam-volatile, but the yields, though usually moderate, compare favourably with those obtained by methods earlier than that described in Part I (Hodgson and Marsden, *J.*, 1944, 22). Neutral aqueous *o*- and *p*-nitrobenzenediazonium sulphate react with sodium nitrite without catalysts, and the yields of *o*- and *p*-dinitrobenzene are unaffected when catalysts are used. The mechanism of the reaction is probably the same as that proposed by Hodgson and Ward (this vol., p. 556) for the reaction of alkaline solutions of diazo-compounds with sodium nitrite. The yields of *o*- and *p*-dinitrobenzenes are 70 and 76% respectively, and compare favourably with the 33—38% and 67—82% respectively recorded by Starkey (*Org. Synth.*, 1939, **19**, 41).

The presence of magnesium, nickel, manganese, copper, aluminium, or zinc sulphate, or of disodium hydrogen phosphate, does not significantly improve the yield, the buffering effect of the salt being largely masked by the large excess of sodium nitrite which keeps the pH of the medium almost neutral.

The best yields are obtained with nitro-amines, indicating that ease of replacement of the diazonium by the nitro-group accompanies greater positivity of the significant carbon atom to which the diazonium group is attached (Hodgson, Leigh, and G. Turner, *J.*, 1942, 744).

In certain cases, *e.g.*, *m*-nitro-*p*-toluidine and *p*-toluidine, the decomposition begins with considerable evolution of nitrous fumes, and the first product to come over during the subsequent steam distillation is one arising from deamination, *e.g.*, *m*-nitrotoluene (ca. 15%) from *m*-nitro-*p*-toluidine, which in this particular case acts as a solvent for the 3 : 4-dinitrotoluene which follows it.

When the neutral solution of the diazonium salt cannot be prepared simply, owing to diazo-oxide formation, so that the solid diazonium sulphate has first to be made (cf. Hodgson and Mahadevan, *J.*, 1947, 325), the diazonium sulphate is sometimes insoluble or slightly soluble in the glacial acetic–sulphuric acid medium then employed, and can be removed by filtration through asbestos; *e.g.*, 4-nitro-1-naphthylamine gave a 10% yield of 4-nitro-1-naphthalenediazonium sulphate in this way.

Since the method of replacing the diazonium- by the nitro-group now given, together with those methods previously described in Parts I (*loc. cit.*), II (*J.*, 1947, 127), and III (*J.*, 1947, 1392), provide the easiest efficient techniques for this purpose, it will be convenient to review here their relative applicability. Where the amine can be diazotised in aqueous medium and where the diazonium salt is stable in neutral solution, the present method provides the simplest technique if the products so obtained are steam volatile, *e.g.*, *o*- and *p*-dinitrobenzene. Alternatively, for the above amines, the diazonium cobaltinitrite can be prepared and decomposed; seldom has difficulty been experienced in preparing the solid cobaltinitrite

(as with *m*-nitro-*p*-toluidine), although the influence of steric hindrance has been indicated in Part III. For certain amines which can be diazotised in concentrated aqueous acid medium but which form diazo-oxides in neutral solution, the technique of Part II for obtaining the cobaltinitrite complex is applicable. Where the amine cannot be diazotised in aqueous medium, or where diazo-oxide formation occurs readily on neutralising the diazonium solution, the method of Part III involving the preparation of solid diazonium sulphates (Hodgson and Mahadevan, *loc. cit.*) and their subsequent decomposition by sodium nitrite–cupro-cupri sulphite can be used. This method cannot be applied, however, when difficulty is experienced in obtaining a good yield of the solid diazonium salt on solvent precipitation from nitrosyl-sulphuric–glacial acetic acid medium, so that, while it is excellent for preparing 1 : 2- or 1 : 4-dinitronaphthalene, it is unsatisfactory when applied to *m*-nitro-*p*-toluidine or *p*-anisidine. Only with amines which must be diazotised in concentrated acid media and whose diazonium sulphates cannot be obtained by the method of Hodgson and Mahadevan (*loc. cit.*), owing to the presence of negative groups rendering the diazonium sulphates very readily soluble in the solvent-acid medium, are suitable methods not yet available.

Since the diazonium group in alkaline and neutral media is so very susceptible towards sodium nitrite with group replacement, while in acid media free nitrous acid is very reactive, especially towards phenols (if present as in diazo-decompositions), it is desirable in all diazotisations to remove any excess of nitrous acid as a precautionary measure before subsequent work.

Experimental.

Preparation of o- *and* p-*Dinitrobenzene.*—*o*- or *p*-Nitroaniline (25 g.) is dissolved in a mixture of sulphuric acid (25 c.c., *d* 1·84) and water (55 c.c.), and the solution cooled to 0° with vigorous stirring to obtain a finely divided precipitate, after which crushed ice (40 g.) is introduced, followed by a solution of sodium nitrite (25 g.) in water (50 c.c.) added in one batch. The mixture is then poured on powdered calcium carbonate (50 g.) and ice, and stirred until frothing ceases, after which it is stirred portionwise into a solution of sodium nitrite (250 g.) in water (200 c.c.) at 50°, and finally heated at 70° for 10 minutes. The *o*- or *p*-dinitrobenzene so formed is steam distilled from the mixture (21·3 g. of *o*- and 23 g. of *p*-dinitrobenzene, *i.e.*, yields of 70% and 76% respectively); the m. p.s of the very pale yellow products are 117° and 173° respectively, indicating that they are pure. Further amounts may be recovered from the cold filtered steam distillate by solvent extraction (*ca.* 3—5%).

General Procedure for Other Amines.—The amine (0·05 g.-mol.) is diazotised with the minimum quantity of dilute hydrochloric or sulphuric acid either in the usual way or as for *p*-nitroaniline above should this process be necessary, and a neutral solution of the diazonium salt is obtained by addition to calcium carbonate at 0°. In the event of the instability of this neutral solution, *e.g.*, with *p*-toluidine and 4-nitro-1-naphthylamine, the neutral mixture is stirred forthwith into a hot solution of sodium nitrite (50 g.) and crystallised copper sulphate (5 g.) in water (50 c.c.) containing cuprous oxide (3 g.) in suspension. In the more stable cases, the neutralised solution is filtered, and either decomposed as above or added to a mixture of cupro-cupri sulphite prepared by addition of an aqueous solution of crystallised sodium sulphite (25 g.) to one of crystallised copper sulphate (25 g.). If the reaction product is volatile in steam, it is removed thereby, and, if not, the hot mixture is filtered from the solid product which is then dried and extracted with a suitable solvent such as ethanol and glacial acetic acid : a typical example is as follows.

Preparation of 1 : 4-*dinitronaphthalene.* 4-Nitro-1-naphthylamine (10 g.) is stirred into a mixture of hydrochloric acid (20 c.c., *d* 1·18) and water (20 c.c.); crushed ice (30 g.) is then added to reduce the temperature to 0°, and then a solution of sodium nitrite (5 g.) in the minimum quantity of water is added in one batch. When diazotisation is complete, the liquor is filtered, the filtrate stirred into calcium carbonate (10 g.), and the neutralised mixture stirred immediately into one of sodium nitrite (75 g.), cuprous oxide (10 g.), crystallised copper sulphate (15 g.), and water (100 c.c.), which is just below the boiling point. Decomposition is immediate, and, after cooling, the solids are filtered off, washed with water, dilute sodium hydroxide, and water, dried, and extracted with boiling ethanol (250 c.c.). The extract is boiled with charcoal, filtered, and concentrated; 1 : 4-dinitronaphthalene (m. p. 128—130°) then crystallises out; yield, 2·9 g. (25%). The compound is obtained pure by one crystallisation from alcohol (charcoal).

Amine diazotised.	Product.	Yield, %.	Remarks.
Aniline	Nitrobenzene	35	Small amount of diphenyl formed.
o-Nitroaniline	*o*-Dinitrobenzene	70	Volatile in steam.
p-Nitroaniline	*p*-Dinitrobenzene	76	,, ,,
m-Chloroaniline	*m*-Chloronitrobenzene	12	,, ,,
p-Chloroaniline	*p*-Chloronitrobenzene	35	,, ,,
m-Toluidine	*m*-Nitrotoluene	13	,, ,,
p-Anisidine	*p*-Nitroanisole	16	,, ,,
o-Nitro-*p*-toluidine	2 : 4-Dinitrotoluene	30	,, ,,
m-Nitro-*p*-toluidine	3 : 4-Dinitrotoluene	45	Nitrous fumes and some deamination.
β-Naphthylamine	β-Nitronaphthalene	15	
Benzidine	4 : 4′-Dinitrodiphenyl	10	Obtained by acetic acid extraction.
4-Nitro-1-naphthylamine	1 : 4-Dinitronaphthalene	25	Obtained by ethanol extraction.
5-Nitro-2-naphthylamine	1 : 6-Dinitronaphthalene	15	,, ,, ,,

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305. *Amidines. Part IX. Preparation of Substituted Amidines from Ketoxime Sulphonates and Ammonia or Amines.*

By P. OXLEY and W. F. SHORT.

Amidines are produced in good yield when ketoxime sulphonates, $CR_2{:}N{\cdot}O{\cdot}SO_2R'$, undergo the Beckmann rearrangement in presence of ammonia or amines, and this confirms Kuhara's view that isomerisation of these oxime esters affords imidosulphonates, $R'{\cdot}SO_2{\cdot}O{\cdot}CR{:}NR$. Iminoethers, $Ar{\cdot}O{\cdot}CR{:}NR$, are similarly produced when an oxime sulphonate is heated with a phenol, and the group $\cdot CR{:}NR$ is also introduced into ethanol, carboxyamides, and sulphonamides by nascent imidosulphonates. When *N*-phenylbenzimidobenzenesulphonate is heated alone it yields benzenesulphonic anhydride and *N*-benzoyl-*NN'*-diphenylbenzamidine, probably as follows: $CPh_2{:}N{\cdot}O{\cdot}SO_2Ph \longrightarrow Ph{\cdot}SO_2{\cdot}O{\cdot}CPh{:}NPh \xrightarrow{(2)} (Ph{\cdot}SO_2)_2O + O(CPh{:}NPh)_2 \longrightarrow Ph{\cdot}CO{\cdot}NPh{\cdot}CPh{:}NPh$. Thermal decomposition of acetoxime benzenesulphonate affords a complicated mixture containing benzenesulphonic anhydride, *NN'*-dimethylacetamidine, and a base $C_8H_{13}N_3$, which is probably a dihydropyrimidine derivative. An excellent yield of 2-ketohexamethyleneimine is obtained by spontaneous rearrangement and hydrolysis of the oxime ester produced by adding benzenesulphonyl chloride to an alkaline solution of *cyclo*hexanone oxime at *ca.* 20°; 2-keto-5-methylhexamethyleneimine is obtained similarly from 4-methyl*cyclo*hexanone.

IN Part III (*J.*, 1947, 382) it was shown that " mixed imides " $R{\cdot}CO{\cdot}NX{\cdot}SO_2Ar$ (X = Alk or Aryl) react with ammonium salts $YZNH_2\}\overset{\ominus}{A}$ to give salts of amidines $R{\cdot}C(:NX){\cdot}NYZ$ and it was suggested that the " mixed imide " isomerises to a sulphonic ester $Ar{\cdot}SO_2{\cdot}O{\cdot}CR{:}NX$ which then undergoes aminolysis. Kuhara, Matsumiya, and Matsunami (*Mem. Coll. Sci. Kyoto*, 1914, **1**, 25, 105) showed that benzophenone oxime benzenesulphonate, m. p. 62°, changed slowly on keeping, more rapidly on exposure to ultraviolet light, and instantaneously on melting, to an unstable oil, readily hydrolysed to benzanilide and benzenesulphonic acid. The absorption spectrum of this oil in alcoholic solution was almost coincident with that of a similar oil obtained from *N*-phenylbenzimidochloride and silver benzenesulphonate, and Kuhara concluded that the rearrangement product of the oxime ester is *N*-phenylbenzimidobenzenesulphonate.

$$\left.\begin{array}{l} Ph_2C{:}N{\cdot}O{\cdot}SO_2Ph \\ \\ Ph{\cdot}SO_2{\cdot}OAg + Cl{\cdot}CPh{:}NPh \end{array}\right\rangle \longrightarrow Ph{\cdot}SO_2{\cdot}O{\cdot}CPh{:}NPh \xrightarrow{H_2O} Ph{\cdot}SO_2{\cdot}OH + Ph{\cdot}CO{\cdot}NHPh$$

Other ketoxime arylsulphonates were subsequently shown to undergo a spontaneous Beckmann rearrangement (Kuhara and Watanabe, *ibid.*, p. 349; Kuhara, Agatsuma, and Araki, *ibid.*, 1917, **3**, 1), but the oily products were not characterised except by hydrolysis. Chapman and Howis (*J.*, 1933, 806) found that the picryl ethers of a number of ketoximes undergo spontaneous rearrangement on heating to give solid compounds in which the picryl group is attached to nitrogen. Thus, benzophenone oxime picryl ether gives benz-*N*-picrylanilide, and Chapman suggested that *N*-phenylbenziminopicryl ether, the first product of the Beckmann change, is too unstable to be isolated since it is immediately transformed into the picrylanilide:

$$Ph_2C{:}N{\cdot}O{\cdot}Pic \longrightarrow PhN{:}CPh{\cdot}O{\cdot}Pic \longrightarrow Pic{\cdot}NPh{\cdot}CO{\cdot}Ph \; [Pic = C_6H_2(NO_2)_3{\cdot}]$$

Chapman rejected the imidosulphonate formulæ assigned by Kuhara to rearrangement products of the oxime sulphonates on the ground that substances having this formula would be unstable, and suggested that they are more probably *N*-acylbenzenesulphonalkylamides or *N*-acylbenzenesulphonanilides, produced from the imidosulphonates by further intramolecular change: $Ph{\cdot}SO_2{\cdot}O{\cdot}CPh{:}NPh \longrightarrow Ph{\cdot}SO_2{\cdot}NPh{\cdot}CO{\cdot}Ph$. The final products obtained from benzophenone oxime benzenesulphonate and the corresponding acetophenone derivative would be *N*-benzoyl- and *N*-acetyl-benzenesulphonanilide and should have solidified since they melt at 114° and 115°, respectively. Moreover, Kuhara, Matsumiya, and Matsunami (*loc. cit.*) state that the oil obtained from the benzophenone derivative hydrolyses slowly in moist air and very rapidly in water, whereas we find that *N*-benzoylbenzenesulphonanilide is not appreciably hydrolysed by

10% aqueous benzenesulphonic acid at 100° in ½ hour, and the other " mixed imides " which we have prepared are also not easily hydrolysed (Part III). At temperatures above 230° the mixed imides reacted with amines to yield a carboxyamide and a sulphonamide, and we suggested that, although ammonium salts were unable to effect this fission, they converted mixed imides into amidinium salts because the imides were partly transformed into the isomeric sulphonic esters on heating :

$$\begin{array}{l} R{\cdot}CO{\cdot}NX{\cdot}SO_2Ar + R'{\cdot}NH_2 \longrightarrow R{\cdot}CO{\cdot}NHR' + Ar{\cdot}SO_2{\cdot}NHX \\ \quad\downarrow \\ Ar{\cdot}SO_2{\cdot}O{\cdot}CR{:}NX \xrightarrow{R'{\cdot}\overset{\oplus}{N}H_2\overset{\ominus}{A}} R{\cdot}C(:NX){\cdot}\overset{\oplus}{N}H_2R'\}Ar\overset{\ominus}{SO_3} + AH \end{array}$$

Since amidines might be formed from the imidosulphonates and ammonia or amines at room temperature, the action of these bases on Kuhara's oily transformation products might decide which of the proposed structures should be assigned to them. We now find that these oils are readily converted into amidines by the action of bases, so that Kuhara's imidosulphonate formula is confirmed. By carrying out the Beckmann rearrangements of the oxime sulphonates in presence of a base and a diluent, the imidosulphonate is immediately converted into an amidinium salt, and, in this way, complications resulting from the readiness with which some imidosulphonates undergo self condensation (see below) are avoided. Intermediates need not be isolated, the ketone being converted successively into the oxime and oxime sulphonate in aqueous alkaline solution, and a dried solution of the oxime ester in benzene, toluene, xylene, or nitrobenzene being heated with the amine, or in a stream of ammonia. The reaction is usually strongly exothermic. Good yields of amidines are customarily obtained from equimolecular quantities of the oxime ester and amine, but when the strength of the amine approaches or exceeds that of the resulting amidine, lower yields of amidines are obtained under these conditions and the product contains compounds produced by the self-condensation of the imidosulphonate. The use of an excess of amine improves the yield of amidine in such cases; for example, the yield of *N*-phenyl-*N'*-benzylbenzamidine obtained from benzophenone oxime benzenesulphonate increases from 42 to 93% when the amount of benzylamine is increased from 1 to 2 molecules. It is evident that the imidosulphonates react with amines in benzene or toluene solution but are unable to react with amine salts under these conditions. (This conclusion is not incompatible with the suggestion that imidosulphonates, produced by isomerisation of mixed imides at *ca.* 200°, yield amidinium salts by reaction with amine salts at that temperature.) Details of the preparation of 28 amidines from 9 ketoximes are given in the Experimental section (see Table) to illustrate the method, and it will be noted that *cyclo*pentanone and *cyclo*hexanone oxime benzenesulphonates afford derivatives of iminopentamethyleneimine and iminohexamethyleneimine respectively.

The order of stability of the oxime benzenesulphonates is represented by the sequence $\ddot{C}Me_2 \gg p\text{-}Br{\cdot}C_6H_4{\cdot}\ddot{C}Me > \ddot{C}Ph_2 > \ddot{C}_5H_8 > Me{\cdot}\ddot{C}Et > Ph{\cdot}\ddot{C}Me \gg \ddot{C}Et_2$ and $\ddot{C}_6H_{10}$; the esters derived from diethyl ketone and *cyclo*hexanone decompose within a few hours at room temperature, those containing the groups occupying the central section of the sequence are somewhat more stable, and acetoxime benzenesulphonate remains unchanged for several weeks at room temperature. The preparation of amidines from acetoxime benzenesulphonate must be carried out in a solvent of higher boiling point, since rearrangement to the imidosulphonate does not occur in boiling benzene. On the other hand, *cyclo*hexanone oxime benzenesulphonate must be prepared below 0°, extracted into a solvent, and the solution used immediately in the preparation of amidines. The instability of this ester may be turned to account in the preparation of 2-ketohexamethyleneimine (" ε-caprolactam ") which is obtained in over 80% yield when benzene- or toluene-*p*-sulphonyl chloride is added to an alkaline solution of *cyclo*hexanone oxime at 20—22°. Under these conditions, the oxime benzenesulphonate rearranges as it is formed and the imidosulphonate is hydrolysed to the lactam. We have prepared 200-g. batches in this way and much larger quantities could probably be prepared in one operation if desired. The method is therefore superior to that in which *cyclo*hexanone oxime is rearranged in presence of 85% sulphuric acid, a reaction which proceeds so violently that only 10-g. portions of oxime can safely be rearranged at once (see *Org. Synth.*, 1934, **14**, 80; 1937, **17**, 60). *2-Keto-5-methylhexamethyleneimine* (83%) is obtained similarly from 4-methyl*cyclo*hexanone.

The production of amidines when a ketoxime sulphonate undergoes the Beckmann transformation in presence of ammonia or of a primary or secondary amine is due essentially to the fact that the intermediate imidosulphonates behave as " alkylating " agents, and this prompted us to examine the behaviour of these esters towards phenols and other compounds

capable of alkylation. High yields of N-*methylacetimido-phenyl* and -p-*tolyl ethers* are obtained when acetoxime benzenesulphonate and phenol or *p*-cresol are boiled in toluene solution: $Ar{\cdot}OH + Ph{\cdot}SO_2{\cdot}O{\cdot}CMe{:}NMe \longrightarrow Ar{\cdot}O{\cdot}CMe{:}NMe,Ph{\cdot}SO_3H$, and *N*-phenylbenzimino-β-naphthyl ether (70—72%) is similarly obtained from benzophenone oxime benzenesulphonate and β-naphthol. The benzophenone derivative and ethyl alcohol in boiling pyridine–benzene give *N*-phenylbenziminoethyl ether (94%) and pyridinium benzenesulphonate. Benzenesulphonamide is similarly " alkylated " by acetoxime benzenesulphonate and by benzophenone oxime benzenesulphonate, the products being N-*benzenesulphonyl*-N′-*methylacetamidine* and *N*-benzenesulphonyl-*N*′-phenylbenzamidine respectively. Acetoxime benzenesulphonate and benzenesulphonmethylamide afford N-*benzenesulphonyl*-NN′-*dimethylacetamidine*. Benzamide and benzophenone oxime benzenesulphonate give only 4·5% of *N*-benzoyl-*N*′-phenylbenzamidine, the main products, phenyl cyanide (80%) and benzanilide (88%), doubtless being formed owing to preponderating *O*-" alkylation " :

$$\begin{array}{ccccccc} Ph{\cdot}N{:}CPh & & Ph{\cdot}SO_2{\cdot}O{\cdot}CPh{:}NPh & & Ph{\cdot}N{:}CPh & & Ph{\cdot}NH{\cdot}CO{\cdot}Ph \\ | & \longleftarrow & + & \longrightarrow & | & \longrightarrow & + \\ Ph{\cdot}CO{\cdot}NH & & Ph{\cdot}CO{\cdot}NH_2 & & Ph{\cdot}C(:NH){\cdot}O & & Ph{\cdot}CN \end{array}$$

When benzophenone oxime benzenesulphonate and aniline are heated in benzene solution, *NN*′-diphenylbenzamidinium benzenesulphonate is obtained in 92·5% yield, but if the benzene solution of the oxime ester is boiled for some time before addition of aniline, the yield of this amidine falls, and if the addition of aniline is delayed for 30 minutes, the sole products of the reaction are phenylammonium benzenesulphonate (0·47 mol.), benzenesulphonanilide (0·47 mol.), and *N*-benzoyl-*NN*′-diphenylbenzamidine (*ca.* 0·5 mol.). When *p*-toluidine is used in place of aniline, the product contains the same benzoyl-amidine, but the phenylammonium salt and the anilide are replaced by the corresponding *p*-toluidine derivatives. These results, and the absence of benzenesulphonic acid in the heated solution of the oxime ester, suggest that one of the transformation products is benzenesulphonic anhydride, and this compound can in fact be isolated from the solution. Similarly, toluene-*p*-sulphonic anhydride is one of the products obtained by the decomposition of benzophenone oxime toluene-*p*-sulphonate. We believe that the correct interpretation of these transformations is expressed in the following scheme, suggested to us by Dr. D. A. Peak :

$$2Ph{\cdot}SO_2{\cdot}O{\cdot}CPh{:}NPh \longrightarrow (Ph{\cdot}SO_2)_2O + Ph{\cdot}N{:}CPh{\cdot}O{\cdot}CPh{:}NPh \quad (I)$$

$$\downarrow$$

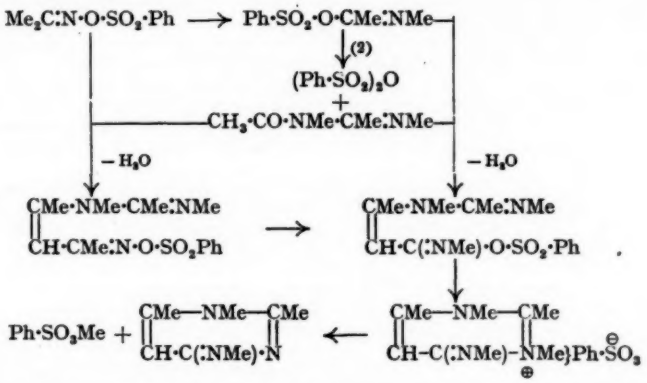
$$O{:}CPh{\cdot}NPh{\cdot}CPh{:}NPh \quad (II)$$

The conversion of the imidosulphonate into benzenesulphonic anhydride and the hypothetical intermediate (I) is analogous to the production of two anhydrides from a mixed anhydride. Analogies for the isomerisation of (I) into the benzoylated amidine (II) are to be found in the rearrangement of 8-alkoxycaffeine to 1 : 3 : 7-trimethyl-9-alkyluric acids (Wislicenus and Körber, *Ber.*, 1902, **35**, 1991; Biltz and Sturfe, *Annalen*, 1916, **413**, 197; Biltz and Bergius, *ibid.*, 1917, **414**, 54), and of *N*-aryl benziminophenyl ethers into the corresponding benzoyldiphenylamines (Chapman, *J.*, 1927, 1743, and earlier papers).* Acetoxime benzenesulphonate does not rearrange in boiling benzene or dichloroethylene but is completely decomposed after 3 hours in boiling toluene, or in ½ hour in boiling xylene, a brown oil separating from the solution. We believe that the initial stages of this decomposition are similar to those which occur in the case of benzophenone oxime benzenesulphonate, but are succeeded by the production of a complex mixture of condensation products, only a small fraction of which has been isolated in the form of pure compounds. The toluene solution contains benzenesulphonic anhydride (8·5—10%)† and methyl benzenesulphonate (4·8%). The brown oil is readily soluble in water to a strongly acid solution, and if this is neutralised with potassium hydroxide and passed through a charcoal column, subsequent elution with water removes potassium benzenesulphonate (43%), *NN*′-dimethylacetamidinium benzenesulphonate (23%), and methylammonium benzenesulphonate (2·7%). The column is then eluted with methanol and saturated aqueous lithium picrate is added to an aqueous solution of the extract, giving the *picrate*, m. p. 166°, of a base $C_8H_{13}N_3$.

* Wislicenus and Goldschmidt (*Ber.*, 1900, **33**, 1467) also state that *N*-phenylformiminomethyl ether, the corresponding ethyl ether, and *N*-phenylbenziminomethyl ether rearrange on heating to give formomethylanilide, formoethylanilide, and benzmethylanilide, respectively, but repetition of the experiments with the formimino-ethers by Grunfeld (*Bull. Soc. chim.*, 1936, **3**, 668) gave entirely different products.

† Yields of the decomposition products of the oxime ester are calculated on the basis of the scheme on p. 1517.

When the reaction is carried out in toluene solution, the yield of picrate is 3·5%, but rises to 6·1% in xylene. The compound $C_8H_{13}N_3$ is a strong mono-acid base, soluble in water, so readily oxidised in air, with production of a pink colour, that a pure specimen could not be prepared, and degradation experiments were therefore carried out with the picrate, *toluene*-p-*sulphonate*, m. p. 166°, or *hydrochloride*, m. p. 222—223° (decomp.). When these salts are boiled with aqueous sodium hydroxide decomposition occurs and the following products are formed: ammonia (1 mol.), methylamine (2 mols.), acetic acid (2·7 mols.), and acetone (*ca.* 0·1 mol.). These results suggest that the base $C_8H_{13}N_3$ is 4-methylimino-1 : 2 : 6-trimethyl-1 : 4-dihydropyrimidine, produced by condensation of *N*-acetyl-*NN'*-dimethylacetamidine, either with *N*-methylacetimidosulphonate or with acetoxime benzenesulphonate. The yield of the base $C_8H_{13}N_3$ is so small that the second alternative cannot be excluded on the ground that at a subsequent stage it involves an intermediate which would probably be the minor product of the postulated Beckmann change. Owing to the slow hydrolysis of benzenesulphonic anhydride, part of the water produced in the condensation would probably be available for hydrolysis of *N*-acetyl-*NN'*-diethylacetamidine to *NN'*-dimethylacetamidine. The production of the *N*-acetylamidine would be analagous to the formation of *N*-benzoyl-*NN'*-diphenylbenzamidine in the rearrangement of benzophenone oxime benzenesulphonate (above), and the fact that none is isolated may be due to its hydrolysis during the reaction and subsequent working up of the products. Wallach (*Ber.*, 1882, **15**, 208) and Heymons (*Ber.*, 1932, **65**, 327) record facile hydrolysis of *N*-acetyl-*NN'*-diethylacetamidine to the amidine.



A trace of *picrate*, m. p. 231°, was isolated from a sample of acetoxime benzenesulphonate which had decomposed at room temperature, and the analytical results suggest that this compound may correspond to the methobenzenesulphonate formulated above.

Acetophenone oxime benzenesulphonate also decomposes exothermically when boiled with benzene, and the product contains *NN'*-diphenylacetamidine (22%), acetanilide (27%), and probably benzenesulphonic anhydride, since benzenesulphonanilide (17·6%) and anilinium benzenesulphonate (26%) are produced on adding aniline. Similarly, *p*-bromoacetophenone oxime benzenesulphonate in boiling benzene affords NN'-*bis*-p-*bromophenylacetamidine* (55%) and, after the addition of *p*-bromoaniline, p-*bromoanilinium benzenesulphonate* (21%) and *benzenesulphon*-p-*bromoanilide* (16%). The behaviour of these two oxime esters is therefore similar to that of acetoxime benzenesulphonate, and the formation of dihydropyrimidine derivatives is not excluded since we did not make an exhaustive examination of the reaction products.

The rearrangement of ketoxime sulphonates in presence of alkylenediamines and hydroxyamines will be described in a subsequent communication.

Experimental.

Preparation of Oxime Benzenesulphonates.

Benzophenone Oxime Benzenesulphonate.—Hydroxylammonium chloride (40 g.; 1·15 mols.) was added to a solution of benzophenone (91 g.) and sodium acetate trihydrate (100 g.; 1·5 mols.) in methanol (400 c.c.), and the mixture, containing precipitated sodium chloride, was boiled for 2 hours, diluted with water (200 c.c.), cooled, and filtered. The benzophenone oxime was washed with 50% aqueous methanol and with water. Yield, 92 g. (93%); m. p. 142·5—143·5°. A solution of the oxime (10 g.) in 1·13N-potassium hydroxide (50 c.c.; 1·1 mols.) and acetone (50 c.c.) was cooled to 10° and stirred during the addition of benzenesulphonyl chloride (9 g.; 1 mol.) at this temperature. After a further 15 minutes'

stirring, the oxime ester was collected, washed successively with 50% aqueous acetone and water, and dried over phosphoric oxide at 1 mm. Rapid drying is essential to prevent decomposition. Yield, 16·4—16·5 g. (96—96·5%); m. p. 78—80° (decomp.). Kuhara, Matsumiya, and Matsunami (*loc. cit.*) give m. p. 62°. This benzenesulphonate decomposed on keeping overnight, and was therefore used in the preparation of amidines as soon as it was dry.

Benzophenone Oxime Toluene-p-*sulphonate.*—A 30% (w/w) acetone solution of toluene-*p*-sulphonyl chloride was added to an alkaline solution of the oxime as in the preceding example. The yield of ester, m. p. 88° (decomp.), was 91%.

Acetophenone Oxime Benzenesulphonate and Toluene-p-*sulphonate.*—Acetophenone (12 g.), hydroxylammonium chloride (7 g.; 1 mol.), and 5N-potassium hydroxide (50 c.c.; 2·5 mols) were stirred vigorously for 15 minutes; the solution was then cooled to 10° and the sulphonyl chloride (18 g. of benzenesulphonyl chloride, or 19·4 g. of toluene-*p*-sulphonyl chloride; 1·02 mols.) was added during 10 minutes at 10—12° with vigorous stirring. After a further 20 minutes' stirring, the crystals were collected, washed with cold water, and dried rapidly over phosphoric oxide in a vacuum. The yield of benzenesulphonate was 23·3—23·5 g. (85%) and that of the toluene-*p*-sulphonate was similar. These esters decomposed on keeping overnight and were invariably used at once in the preparation of amidines. Preparations carried out in aqueous acetone afforded less crystalline oxime esters.

p-*Bromoacetophenone Oxime Benzenesulphonate.*—*p*-Bromoacetophenone (38 g.) (*Org. Synth.*, 1925, **5**, 17), hydroxylammonium chloride (14 g.; 1·06 mol.), sodium acetate trihydrate (28 g.; 1·07 mols.), and methanol (100 c.c.) were boiled under reflux for 30 minutes, then diluted with water (100 c.c.) and cooled. The oxime was collected, washed with 50% aqueous methanol (100 c.c.), and dried. Yield, 34 g. (84%); m. p. 129°. Collet (*Bull. Soc. chim.*, 1899, **21**, 67) records m. p. 128—128·5°. Benzenesulphonyl chloride (9 g.; 1·02 mols.) was added to a stirred solution of the oxime (10·7 g.) in 1·2N-potassium hydroxide (50 c.c.; 1·2 mols.) and acetone (25 c.c.). The oxime ester separated as an oil which soon solidified and, after an additional 30 minutes' stirring, the solid was collected, washed with 30% aqueous acetone, and dried quickly in a vacuum desiccator over phosphoric oxide. Yield, 16·8 g. (95%); m. p. 103° (decomp.). The benzenesulphonate decomposes within 48 hours at room temperature.

cyclo*Pentanone Oxime Benzenesulphonate.*—*cyclo*Pentanone (8·4 g.) was added to a stirred solution of hydroxylammonium chloride (7 g.; 1 mol.) in 4·5N-potassium hydroxide (50 c.c.; 2·25 mols.), previously cooled to 0°, and the mixture was stirred for 40 minutes to complete the reaction. Acetone (10 c.c.) was then added, followed by benzenesulphonyl chloride (18 g.; 1·02 mols.) which was introduced below 0°, and after an additional 30 minutes' stirring below 0°, the oxime ester was extracted with benzene or nitrobenzene. The solution was washed with water, dried quickly ($MgSO_4$), and used immediately in the preparation of amidines.

cyclo*Hexanone Oxime Benzenesulphonate.*—The proportions of reactants were the same as those employed in the preparation of 2-ketohexamethyleneimine (below), but the benzenesulphonyl chloride was introduced below − 10° and the oxime ester was immediately collected in benzene or nitrobenzene. The solution was dried ($MgSO_4$) and used at once in the preparation of amidines.

Acetoxime Benzenesulphonate.—Hydroxylammonium chloride (7 g.) was added to a mixture of acetone (20 c.c.; 2·7 mols.) and 5N-sodium hydroxide (50 c.c.; 2·5 mols.), and after an additional 10 minutes' stirring, the solution was cooled to 10° and benzenesulphonyl chloride (17·65 g.; 0·9 mol.) was slowly introduced at 10—12°. The ester, which quickly solidified, was collected after an hour, washed thoroughly with water and dried (P_2O_5). Yield, 21·0 g. (98% on the hydroxylamine); m. p. 53° (Found: N, 6·6. Calc. for $C_9H_{11}O_3NS$: N, 6·6%). Wege (*Ber.*, 1891, **24**, 3538) records m. p. 52·5° (see also Kuhara, Agatsuma, and Araki, *loc. cit.*). This oxime benzenesulphonate could be kept for several weeks without apparent decomposition, but complete decomposition occurred in 3 months in summer. The examination of the products of decomposition is described below.

Methyl Ethyl Ketoxime Benzenesulphonate.—Benzenesulphonyl chloride (44 g.; 1 mol.) was added dropwise below 20° to a vigorously stirred solution of methyl ethyl ketoxime (22 g.) in 2·6N-sodium hydroxide (105 c.c.; 1·1 mols.), and the mixture was then stirred at 5—10° for an hour. The oxime ester was collected in benzene, and the solution washed with water, dried (K_2CO_3), and evaporated to constant weight below 20° in a vacuum, leaving methyl ethyl ketoxime benzenesulphonate as a pale yellow oil (46·6—49·3 g.; 81·2—85·9%).

Diethyl Ketoxime Benzenesulphonate.—A solution of diethyl ketoxime, prepared from diethyl ketone (17·2 g.), hydroxylammonium chloride (14 g.; 1·01 mols.), and 5N-sodium hydroxide (80 c.c.; 2 mols.), was cooled to 0° and stirred during the addition of benzenesulphonyl chloride (35·3 g.; 1 mol.). Acetone (25 c.c.) was then added, and the mixture stirred at 0° for 25 minutes, and extracted with benzene (100 c.c.). The solution was quickly dried (K_2CO_3) and used immediately in the preparation of amidines. This oxime ester is unstable and complete decomposition occurred in an experiment in which the reaction mixture was stirred at 5° for 2 hours.

*Preparation of 2-Ketohexamethyleneimines.**

2-*Ketohexamethyleneimine.*—Hydroxylammonium chloride (140 g.; 1 mol.) was added during 5—10 minutes to a stirred suspension of *cyclo*hexanone (196 g.) in 5·75N-sodium hydroxide (1050 c.c.; 3 mols.), the solution was cooled in running water, and benzenesulphonyl chloride (353 g.; 1 mol.) was added slowly so that there was no accumulation of oil in the mixture and the temperature remained at 22—23°. This addition required *ca.* 1 hour, and after the mixture had been stirred for an additional 10—15 minutes, sodium benzenesulphonate was removed by filtration. The filtrate, which was faintly alkaline to phenolphthalein, was extracted with chloroform (400 c.c. and 7 × 200 c.c.) and the dried solution ($MgSO_4$) was evaporated. Distillation of the residue afforded recovered *cyclo*hexanone (7 g.) and then 2-ketohexamethyleneimine (181—184 g.; 80—81·4%), b. p. 148—149°/16 mm. The crude lactam had m. p. 64—66°, raised by recrystallisation from light petroleum (b. p. 60—80°) to 68·5°. When toluene-*p*-sulphonyl chloride (1 mol.) was substituted for benzenesulphonyl chloride, it was dissolved in the

* See also B.P. 577,696.

minimum of acetone and this solvent was removed before the chloroform extraction; the yield of lactam was 80%. The use of methanesulphonyl chloride in an experiment on a 0·1 g.-molar scale and in which the mixture was stirred at 0° for 3 hours after the addition of the chloride resulted in a 41% recovery of *cyclo*hexanone and a 45% yield of the lactam.

2-Keto-5-methylhexamethyleneimine.—A suspension of 4-methyl*cyclo*hexanone (112 g.) in 5N-potassium hydroxide (600 c.c.; 3 mols.) was cooled in water at *ca.* 10°, and stirred during the addition of hydroxylammonium chloride (70 g.; 1 mol.) at such a rate that the temperature remained at 24—25°. After stirring for a further 5 minutes, benzenesulphonyl chloride (177 g.; 1 mol.) was added during 60—65 minutes, so that the temperature was kept at 20—22°, and stirring was continued for a further 15 minutes. The products, isolated as described in the preceding example, consisted of unchanged 4-methyl*cyclo*hexanone, b. p. *ca.* 80°/3 mm. (3 g.; 2·6%), *2-keto-5-methylhexamethyleneimine*, b. p. 116°/1·5 mm. (105 g., 82·7%), and a dark brown distillation residue (14·2 g.). The lactam slowly solidified to deliquescent crystals, m. p. 43° (Found: N, 10·9. $C_7H_{13}ON$ requires N, 11·0%). Wallach (*Annalen*, 1906, **346**, 252), who obtained this lactam as a liquid by the rearrangement of 4-methyl*cyclo*hexanone oxime in presence of sulphuric acid, records no analysis but analysed a hydrochloride of unstated m. p.

*Preparation of Amidines from Oxime Sulphonates.**

In order to avoid repetition, the amidines have been collected in a table, and a general method of preparation is described below. Slight variations from the general method are recorded in footnotes to the table, and detailed descriptions are given below in cases where special features are involved.

The experiments were conducted with 0·05—0·2 g.-mol. of oxime sulphonate, prepared as described above, and the yields of amidine are calculated on the the oxime ester, or, when this was not isolated, on the ketone employed in making the oxime. It is to be understood that identification of known compounds included not merely the determination of the m. p. as quoted, but also comparison with an authentic specimen. Except in cases where the amine approaches the resulting amidine in basic strength (see below), the oxime sulphonate and the amine were employed in equimolecular proportion, the amine being added to a benzene solution of the oxime ester containing 0·1—0·2 g.-mol. per 100 c.c. The solution was warmed on the steam-bath to start an exothermic reaction which caused the solution to boil spontaneously for several minutes, and the amidinium sulphonate usually separated almost completely, either as a crystalline solid (Nos. 5, 6, 7, 8, 14, and 15), or as a heavy water-soluble oil. In the case of acetoxime sulphonates, which are exceptionally stable, it was necessary to use toluene as solvent in place of benzene and the solution was preferably heated over a gauze. The reaction was completed in all cases by boiling under reflux for a further 15—30 minutes. When the amidinium sulphonate separated as a solid, it was collected from the cold reaction product and purified by crystallisation from water or alcohol. If the amidinium salt did not crystallise readily, the free amidine was liberated from the reaction product by shaking with cold 2—3N-sodium hydroxide, and the benzene or toluene solution was then separated, dried (K_2CO_3), and concentrated. The residue of amidine was then purified by distillation, sublimation, or crystallisation.

When the basic strength of the amine approaches or exceeds that of the resulting amidine, 2 mols. of amine should be used. In these cases (Nos. 2, 3, 4, and 11), the solid deposited from the reaction mixture was the substituted ammonium sulphonate and the free amidine remained in the benzene solution The substituted ammonium sulphonate was removed by filtration, or by shaking with water, and the amidine was then isolated from the benzene solution in the usual way.

In the preparation of *N*-monosubstituted amidines (Nos. 1, 15, 17, and 19) the benzene or toluene solution of the oxime sulphonate was saturated with dry ammonia and a stream of the gas was passed into the solution throughout the reaction. Some ammonium sulphonate was also formed in these cases. Nitrobenzene was used as solvent in two of these experiments (Nos. 15 and 17).

N-Phenyl-N′-benzylbenzamidine.—(1) A solution of benzophenone oxime benzenesulphonate (16·5 g.) and benzylamine (5·4 g.; 1 mol.) in dry benzene (100 c.c.) was boiled for ½ hour, cooled, and filtered from the solid which had separated. The crystals (6 g., or 47%) consisted of benzylammonium benzenesulphonate, m. p. and mixed m. p. 193° (see below). The benzene solution was shaken with 2N-sodium hydroxide, dried, and evaporated, giving a partially crystalline residue (12·95 g.), which was boiled with light petroleum (b. p. 80—100°). The solution was decanted from a dark, sparingly soluble gum, and the yellow needles (3 g.) which separated on cooling afforded benzanilide (0·7 g.), m. p. and mixed m. p. 163°, after recrystallisation from benzene. The residue obtained by removing the benzene gave *N*-phenyl-*N′*-benzylbenzamidine (2 g., 14·3%), m. p. 100°, after recrystallisation from *iso*propanol (see table). The light petroleum was removed from the filtrate from the benzanilide and the residue was dissolved in a solution of picric acid (5 g.) in *iso*propanol (50 c.c.). The gummy precipitate crystallised on keeping overnight, and about half of this redissolved on warming, leaving a very sparingly soluble picrate (3·1 g., m. p. 215—218°), which afforded *NN′*-diphenylbenzamidinium picrate, m. p. and mixed m. p. 220—221°, on recrystallisation from *iso*propanol. The original *iso*propanol solution slowly deposited *N-phenyl-N′-benzylbenzamidinium picrate* (3·9 g.), m. p. 151°, on cooling (see table for analysis). The total yield of *N*-phenyl-*N′*-benzylbenzamidine was 42%. Benzylammonium benzenesulphonate, obtained from its constituents in *iso*propanol, had m. p. 183—184°, but on recrystallisation or keeping the m. p. rose to 193° (Found: N, 5·2. Calc. for $C_{13}H_{15}O_3NS$: N, 5·3%). Neber and Uber (*Annalen*, 1928, **467**, 60) give the m. p. of this salt as 186° and Schlegel (*Ber.*, 1931, **64**, 1743) gives m. p. 190°. The m. p. of the benzylammonium benzenesulphonate obtained in the amidine preparation was not depressed on admixture with either the high- or the low-melting form prepared from the amine and the acid. (2) When the experiment was repeated using 10·7 g. of benzylamine (2 mols.), the amount of benzylammonium benzenesulphonate precipitated was 12·3 g. (48%), and evaporation of the benzene solution gave a pale yellow oil (14·8 g.) which rapidly solidified to crystals, m. p. 90—95°. Recrystallisation from *iso*propanol or light petroleum (b. p. 100—120°) afforded *N*-phenyl-*N′*-benzylbenzamidine, m. p. 100° (13 g., 93%).

* See also B.PP. 577,478; 579,303.

	Amidine.						Picrate.			
	Yield, %.	M. p.	B. p.	Formula.	Found: N, %.	Req.: N, %.	M. p.	Formula.	Found: N, %.	Req.: N, %.
Amidines from Benzophenone Oxime Benzenesulphonate.										
(1) *N*-Phenylbenzamidine	18	116°	—	—	—	—	148·5°	$C_{19}H_{15}O_7N_5$	16·5	16·5
(2) *N*-Phenyl-*N'*-benzylbenzamidine	93	100	—	—	—	—	151	$C_{26}H_{21}O_7N_5$	13·7	13·6
(3) *N*-Phenyl-*NN*-diethylbenzamidine	90	—	148°/2 mm.	$C_{17}H_{20}N_2$	11·2	11·1	110·5	$C_{23}H_{23}O_7N_5$	14·5	14·5
(4) N'-*Phenyl*-NN-*pentamethylenebenzamidine*	89	—	164/1 mm.	$C_{18}H_{20}N_2$	10·6	10·6	180·5—181	$C_{24}H_{23}O_7N_5$	14·6	14·2
(5) *NN'*-Diphenylbenzamidine	92·5	146·5—147	—	—	—	—	221	—	—	—
(6) *N*-Phenyl-*N'*-*p*-tolylbenzamidine	*ca.* 100	133—135	—	—	—	—	195	—	—	—
(7) *N*-Phenyl-*N'*-*o*-chlorophenylbenzamidine	94	113	—	$C_{19}H_{15}N_2Cl$	9·1	9·1	176·5	$C_{25}H_{18}O_7N_5Cl$	13·2	13·1
(8) *N*-Phenyl-*N'*-*p*-chlorophenylbenzamidine	96	122	—	$C_{19}H_{15}N_2Cl$	9·1	9·1	174	$C_{25}H_{18}O_7N_5Cl$	13·0	13·1
(9) *NN'*-Diphenyl-*N*-methylbenzamidine	90	61	177—179/1 mm.	$C_{20}H_{18}N_2$	10·0	9·8	191 (decomp.)	$C_{26}H_{21}O_7N_5$	13·4	13·6
(10) *NNN'*-Triphenylbenzamidine	83	170	—	—	—	—	116—122	$C_{31}H_{27}O_7N_5$	11·8	12·1
(11) N-*Phenyl*-N'-2-*pyridylbenzamidine*	20	138	—	$C_{18}H_{15}N_3$	15·6	15·4	208	$C_{24}H_{18}O_7N_6$	16·8	16·7
Amidine from Acetophenone Oxime Methanesulphonate.										
(12) *NN'*-Diphenylacetamidine	24	131	—	—	—	—	171	—	—	—
Amidine from Acetophenone Oxime Toluene-p-sulphonate.										
(13) *NN'*-Diphenylacetamidine	95	132·5	—	—	—	—	171	—	—	—
Amidine from p-Bromoacetophenone Oxime Benzenesulphonate.										
(14) NN'-*Bis*-4-*bromophenylacetamidine*	96	129·5	—	$C_{14}H_{12}N_2Br_2$	7·5	7·6	209·5	$C_{20}H_{15}O_7N_5Br_2$	12·2	11·9
Amidines from cycloPentanone Oxime Benzenesulphonate.										
(15) 2-Iminopentamethyleneimine	47	—	—	—	—	—	210—210·5	$C_{11}H_{13}O_7N_5$	21·3	21·4
(16) 2-*Anilopentamethyleneimine*	57·5	93	—	$C_{11}H_{14}N_2$	16·35	16·1	168	$C_{17}H_{17}O_7N_5$	17·5	17·4
Amidines from cycloHexanone Oxime Benzenesulphonate.										
(17) 2-Iminohexamethyleneimine	*ca.* 50	—	—	—	—	—	195·5—196·5	$C_{12}H_{15}O_7N_5$	20·6	20·5
(18) 2-*Anilohexamethyleneimine*	72	105	—	$C_{12}H_{16}N_2$	15·0	14·9	152·5	$C_{18}H_{19}O_7N_5$	17·05	16·8
Amidines from Acetoxime Benzenesulphonate.										
(19) *N*-Methylacetamidine	21	—	—	—	—	—	170·5	$C_9H_{11}O_7N_5$	23·4	23·3

(20) N-cyclo*Hexyl*-N'-*methylacet*-	75	111	—	$C_9H_{18}N_2$	18·1	18·2	119	$C_{15}H_{20}O_7N_5$	18·4	18·3

(20) N-cyclo*Hexyl*-N′-*methylacetamidine*	75	111	—	$C_9H_{18}N_2$	18·1	18·2	119	$C_{15}H_{21}O_7N_5$	18·4	18·3
(21) N-*Phenyl*-N′-*methylacetamidine*	84·5	72	101—102/1 mm.	$C_9H_{12}N_2$	19·0	18·9	119	$C_{15}H_{15}O_7N_5$	18·8	18·6
(22) N-2-*Pyridyl*-N′-*methylacetamidine*	84	—	112/1 mm.	$C_8H_{11}N_3$	27·8	28·2	201	$C_{14}H_{14}O_7N_6$	22·25	22·2
(23) N-2-*Furfuryl*-N′-*methylacetamidine*	69	—	94/1 mm.	$C_8H_{12}ON_2$	18·4	18·4	92·5—93	$C_{14}H_{15}O_8N_5$	18·4	18·4
(24) *NN*-3-Oxapentamethylene-*N*′-methylacetamidine [4-(1′-Methyliminoethyl)morpholine]	40	—	104—106/12 mm.	—	—	—	124	$C_{13}H_{17}O_8N_5$	19·1	18·85
(25) *NN*-Diphenyl-*N*′-methylacetamidine	82	103	150—152/3 mm.	$C_{15}H_{16}N_2$	12·65	12·5	163·5—164	$C_{21}H_{19}O_7N_5$	15·5	15·45
Amidine from Methyl Ethyl Ketoxime Benzenesulphonate.										
(26) N-cyclo*Hexyl*-N′-*ethylacetamidine*	74	71	82—85/1·5 mm.	$C_{10}H_{20}N_2$	16·6	16·7	103—104	$C_{16}H_{22}O_7N_5$	17·5	17·6
Amidines from Diethyl Ketoxime Benzenesulphonate.										
(27) *NNN*′-Triethylpropionamidine	80	—	77—78/17 mm.	—	—	—	77	$C_{15}H_{23}O_7N_5$	18·1	18·2
(28) N-cyclo*Hexyl*-N′-*ethylpropionamidine*	78	—	84—85/1·5 mm.	$C_{11}H_{22}N_2$	15·2	15·4	—	—	—	—

(1) The m. p. of the amidine agrees with the value recorded in the literature (Part I).

(2) Details of this preparation are recorded below. The amidine had the recorded m. p. (Pechmann and Heinze, *Ber.*, 1897, **30**, 1787).

(3) Von Braun (*Ber.*, 1904, **37**, 2682) states that the base has b. p. 188—189°/10 mm., and records m. p. 114° for the picrate. The *benzenesulphonate* had m. p. 160° (Found: N, 6·85. $C_{22}H_{24}O_3N_2S$ requires N, 6·8%).

(4) Von Braun (*ibid.*, p. 2679) records m. p. 174—175° for the picrate. The *benzenesulphonate* had m. p. 160° (Found: N, 6·8. $C_{24}H_{26}O_3N_2S$ requires N, 6·6%).

(5) The m. p. of *NN*′-diphenylbenzamidine is given as 147° in Part I.

(6) The amidine crystallised from *iso*propanol in colourless needles which melted at 133° to an opaque gum, clearing 135°. Ley and Holzweissig (*Ber.*, 1903, **36**, 23) state that the amidine has m. p. 135—136°, with sintering at 133·5°. The m. p. of the picrate agrees with that recorded by Marckwald (*Annalen*, 1895, **286**, 356). The *benzenesulphonate* had m. p. 143° (Found: N, 6·5. $C_{26}H_{24}O_3N_2S$ requires N, 6·3%).

(7) Shah and Heeramaneck (*J.*, 1936, 430) state that *N*-phenyl-*N*′-*o*-chlorophenylbenzamidine has m. p. 113—114°. The *benzenesulphonate* had m. p. 146° (Found: N, 5·9. $C_{25}H_{21}O_3N_2ClS$ requires N, 6·0%).

(8) Joshi, Khanolkar, and Wheeler (*J.*, 1936, 795) state that *N*-phenyl-*N*′-*p*-chlorophenylbenzamidine has m. p. 120—122°. The *benzenesulphonate* had m. p. 180° (Found: N, 6·1. $C_{25}H_{21}O_3N_2ClS$ requires N, 6·0%).

(9) Von Braun and Weissbach (*Ber.*, 1932, **65**, 1574) state that the base has m. p. 47°, b. p. 226—228°/13 mm., and affords a picrate, m. p. 189°.

(10) The m. p. of the amidine agrees with that recorded by v. Braun (*loc. cit.*). The m. p. of the picrate was not improved by recrystallisation.

(11) and (12). These experiments are described in detail below.

(13) *NN*′-Diphenylacetamidinium chloride crystallised from *iso*propanol in colourless prisms, m. p. 224° (decomp.); Maybery and Krause (*Ber.*, 1889, **22**, 3305) record m. p. 214—215° (decomp.).

(14) The *benzenesulphonate* had m. p. 167·5° (Found: N, 5·2. $C_{20}H_{19}O_3N_2Br_2S$ requires N, 5·3%).

(15) Details of this preparation are given below. The *benzenesulphonate* had m. p. 130—131° (Found: N, 11·0. $C_{11}H_{16}O_3N_2S$ requires N, 10·9%). Grave (*J. Amer. Chem. Soc.*, 1924, **46**, 1468) states that the picrate has m. p. 209—210° (corr.).

(16) The *hydrochloride* formed colourless needles, m. p. 169·5—170·5° (Found: N, 13·4. $C_{11}H_{15}N_2Cl$ requires N, 13·3%).

(17) The *benzenesulphonate* had m. p. 133° (Found: N, 10·3. $C_{12}H_{18}O_3N_2S$ requires N, 10·4%).

(18) The *hydrochloride* separated from 95% aqueous-acetone in plates, m. p. 185—186° (Found: N, 12·8. $C_{12}H_{17}N_2Cl$ requires N, 12·5%).

(19) The m. p. of the *picrate* was undepressed on admixture with a specimen prepared from the amidine obtained from *N*-methylacetiminophenyl ether and ammonium benzenesulphonate (see below).

(20) The preparation of *N-cyclo*hexyl-*N*′-methylacetamidinium chloride, m. p. 263—264° (decomp.), from *N*-methylacetimino-*p*-tolyl ether is described below.

(26) N-cyclo*Hexyl*-N′-*ethylacetamidinium chloride* separated from *iso*propanol–ether as a deliquescent powder, m. p. 130—131° (Found: N, 13·7. $C_{10}H_{21}N_2Cl$ requires N, 13·7%). The structure assigned to this amidine is based on its conversion into 2-methyldihydroglyoxaline (described in a subsequent communication). There were indications that a small quantity of the isomeric *N-cyclo*hexyl-*N*′-methylpropionamidine was formed but this could not be isolated pure.

(28) N-cyclo*Hexyl*-N′-*ethylpropionamidinium chloride* had m. p. 113° (Found: N, 12·8. $C_{11}H_{23}N_2Cl$ requires N, 12·8%).

N-*Phenyl*-N′-2-*pyridylbenzamidine*.—There was an exothermic reaction when benzophenone oxime benzenesulphonate (16·5 g.) and 2-aminopyridine (9·4 g.; 2 mols.) were heated in solution in benzene (100 c.c.), and a pale yellow gum separated. After 30 minutes' boiling, the mixture was cooled and the crystals (12·4 g., m. p. 99—100°) were collected. Recrystallisation from acetone afforded 2-*aminopyridinium benzenesulphonate*, m. p. 101° (Found: N, 11·0. $C_{11}H_{12}O_3N_2S$ requires N, 11·1%). The benzene solution was shaken with 2N-sodium hydroxide and evaporated, giving a yellow gum (13·5 g.) which was dissolved in 50 c.c. of benzene–light petroleum (1 : 1). The solution deposited 2·7 g. (20%) of crude amidine, m. p. 135°, which was recrystallised from *iso*propanol giving N-*phenyl*-N′-2-*pyridylbenzamidine*, m. p. 138° (Found: C, 78·3, 78·2; H, 5·35, 5·4; N, 15·6. $C_{18}H_{15}N_3$ requires C, 79·1; H, 5·5; N, 15·4%). The *picrate* separated from methanol in orange plates, m. p. 208° (for analysis, see table). The benzene–petroleum mother liquors afforded a gum (10·8 g.) on evaporation and this was digested with water (50 c.c.) and sufficient benzenesulphonic acid to make the liquid acid to Congo-red. After being cooled, the aqueous layer was decanted from a gum (14 g.), made alkaline, and extracted with ether to give 2-aminopyridine (1·5 g., 16%), m. p. and mixed m. p. 55°, further identified as the picrate, m. p. and mixed m. p. 223°. The gum was dissolved in hot *iso*propanol (20 c.c.), and the cooled solution slowly deposited *NN*′-diphenylbenzamidinium benzenesulphonate, m. p. and mixed m. p. 216—218° (4 g., 38%).

NN′-*Diphenylacetamidine*.—Acetophenone (12 g.) was added at 20° with stirring to a solution prepared from hydroxylamine hydrochloride (7 g., 1 mol.) and 4N-potassium hydroxide (60 c.c., 2·4 mols.), and, after cooling to − 5°, methanesulphonyl chloride (11·5 g.; 1 mol.) was added during 20 minutes. After being stirred for a further 20 minutes, the mixture was extracted with benzene (50 and 25 c.c.), and the dried solution ($MgSO_4$) was added to aniline (10 g.; 1·1 mols.). There was an exothermic reaction on warming, and, after 5 minutes' boiling, the mixture was cooled and filtered from a solid, m. p. 212—214° (4·3 g., 23%), which afforded *phenylammonium methanesulphonate*, m. p. 220°, on recrystallisation from methanol (Found: N, 7·4. $C_7H_{11}O_3NS$ requires N, 7·4%). The benzene solution was extracted successively with acid and alkali. The acid extract was diluted with alcohol (to retain aniline in solution) and made alkaline, giving *NN*′-diphenylacetamidine (5 g., 24%), m. p. 131°. The m. p. of this amidine is recorded as 132·5° in Part III (*J.*, 1947, 382) and as 133·5—134° in Part VII (*J.*, 1947, 1114). Acidification of the alkaline extract gave methanesulphonanilide (1·6 g., 9·3%), m. p. 98—99°, raised to 100—101° by recrystallisation from aqueous alcohol; Duguet (*Rec. Trav. chim.*, 1902, **21**, 76) and Marvel, Helfrick, and Belsley (*J. Amer. Chem. Soc.*, 1929, **51**, 1273) record m. p. 99° and 100·5°, respectively. The low yield of amidine may be due to incomplete esterification of the oxime or to rapid decomposition of the oxime ester.

2-*Iminopentamethyleneimine*.—A solution of *cyclo*pentanone oxime benzenesulphonate in nitrobenzene (75 c.c.), prepared from *cyclo*pentanone (8·4 g.) in the manner already described, was saturated with dry ammonia and warmed. An exothermic reaction took place at 80°, the temperature rising to 150°. The dark solution was allowed to cool in an atmosphere of ammonia, and the crystals (12·1 g., 47%; m. p. 120—125°) which separated were recrystallised from *iso*propanol, giving colourless plates of 2-iminopentamethyleneimine benzenesulphonate, m. p. 130—131°, already described in the table, which also records the properties of the corresponding picrate.

The Reaction of Phenols, Ethanol, Sulphonamides, and Benzamide with Oxime Sulphonates.

N-*Methylacetiminophenyl Ether*.—A heavy oil, which solidified on cooling, was deposited when a solution of acetoxime benzenesulphonate (21·3 g.) and phenol (9·4 g.; 1 mol.) in dry toluene (50 c.c.) was boiled under reflux for an hour. The hygroscopic crystals (28·5 g.), m. p. 127—130°, probably consisted of the benzenesulphonate of the iminoether but could not be purified owing to the ease with which they were hydrolysed, giving phenol and methylammonium benzenesulphonate, m. p. and mixed m. p. 165—166°. When the whole reaction product was shaken with 3N-sodium hydroxide, the crystals disappeared, and distillation of the residue obtained by concentrating the dried toluene solution afforded N-*methylacetiminophenyl ether*, b. p. 65°/1·2 mm. (Found: N, 9·5. $C_9H_{11}ON$ requires N, 9·4%). The yield was 13·2 g. (89%). The *picrate* separated from ether in needles, m. p. 147—149°, but could not be recrystallised without decomposition (Found: N, 15·0. $C_{15}H_{14}O_8N_4$ requires N, 14·8%). A homogeneous solution was produced and heat was evolved when ammonium benzenesulphonate (8·75 g.; 1 mol.) was added to the imino-ether (7·45 g.). Addition of 2M-lithium picrate (25 c.c.) to a solution of the product in water (25 c.c.) produced *N*-methylacetamidinium picrate (10·9 g., 73%), which crystallised from *iso*propanol in needles, m. p. 170·5°, undepressed on admixture with a specimen prepared from acetoxime benzenesulphonate (see table, No. 19).

N-*Methylacetimino*-p-*tolyl Ether*.—Acetoxime benzenesulphonate (10·65 g.) and *p*-cresol (5·4 g.) in toluene (25 c.c.) similarly afforded N-*methylacetimino*-p-*tolyl ether*, b. p. 70—71°/0·5 mm. (Found: N, 8·8. $C_{10}H_{13}ON$ requires N, 8·6%) (7·3 g., 90%). The picrate, m. p. 103—104·5°, was too unstable for complete purification, and the *hydrochloride* was obtained as a colourless powder, m. p. 177° (decomp.) (Found: N, 7·3. $C_{10}H_{14}ONCl$ requires N, 7·0%), by passing hydrogen chloride into an ethereal solution of the base. When this hydrochloride (10 g.) was slowly added to a solution of *cyclo*hexylamine (5 g.; 1 mol.) in dry ethanol (50 c.c.) heat was evolved and a clear solution was formed. After this had been kept overnight, dry ether (100 c.c.) was added and the resulting crystals of N-cyclo*hexyl*-N′-*methylacetamidinium chloride* (9·28 g., 97·5%) had m. p. 263—264° (decomp.), unchanged by recrystallisation from *iso*propanol (Found: N, 14·85. $C_9H_{19}N_2Cl$ requires N, 14·7%).

N-*Benzenesulphonyl*-N′-*methylacetamidine*.—Equimolecular quantities of acetoxime benzenesulphonate (21·3 g.), benzenesulphonamide (15·7 g.), and pyridine (7·9 g.) reacted exothermically when warmed in solution in dry toluene (50 c.c.). A heavy oil separated and, after boiling for ½ hour, the mixture was cooled, made alkaline with 2N-sodium hydroxide, and the dried toluene solution was evaporated. The residual brown oil (11·2 g.) slowly solidified to crystals, m. p. 100—115°, and recrystallisation from water (charcoal) afforded needles (7·5 g., 35%) of N-*benzenesulphonyl*-N′-*methylacetamidine*, m. p. 128° (Found: C, 50·85; H, 5·6; N, 13·1. $C_9H_{12}O_2N_2S$ requires C, 50·95; H, 5·7; N, 13·2%). In contrast to

N-benzenesulphonyl-*N'*-phenylbenzamidine (see below) this compound is a feeble base, soluble in concentrated hydrochloric acid but insoluble in 5N-sodium hydroxide.

N-*Benzenesulphonyl*-NN'-*dimethylacetamidine*.—This compound was prepared from acetoxime benzenesulphonate (42·6 g.) and benzenesulphonmethylamide (34·2 g.; 1 mol.) in toluene solution in the same way as the monomethyl compound. The crude product (23·7 g.) was distilled, giving N-*benzenesulphonyl*-NN'-*dimethylacetamidine* (19·6 g., 43%) as a pale yellow oil, b. p. 139—141°/1 mm. (Found: N, 12·6. $C_{10}H_{14}O_3N_2S$ requires N, 12·4%). This compound was an unexpectedly strong base, its aqueous solution being alkaline to Titan-yellow, and was rapidly hydrolysed by boiling water. The base and an equivalent of picric acid in alcoholic solution afforded the *picrate* as a crystalline powder, m. p. 122° (Found: N, 15·5. $C_{16}H_{17}O_9N_5S$ requires N, 15·4%). A solution of this salt in boiling methanol deposited methylammonium picrate, m. p. and mixed m. p. 210° (decomp.), on cooling.

N-*Phenylbenziminophenyl Ether*.—There was an exothermic reaction when benzophenone oxime benzenesulphonate (16 g.) and phenol (4·5 g.) were warmed in dry benzene (50 c.c.) and the mixture almost solidified. After boiling for 10 minutes, the crystals were collected and washed with dry benzene; then having m. p. 169—170° (decomp.) (19·8 g., or 97%). These crystals, which probably consisted of a benzenesulphonate, were too unstable to recrystallise and were therefore decomposed with 2N-sodium hydroxide in presence of chloroform, giving *N*-phenylbenziminophenyl ether, which crystallised from *iso*propanol or light petroleum (b. p. 100—120°) in prisms, m. p. 104·5—105° (Found: N, 5·2. Calc. for $C_{19}H_{15}ON$: N, 5·1%). Hantzsch (*Ber.*, 1893, **26**, 927) and Chapman (*J.*, 1925, **127**, 1996) record m. p. 104° and 105°, respectively.

N-*Phenylbenzimino-β-naphthyl Ether*.—The unstable benzenesulphonate, m. p. 140—142°, of this ether was obtained in 72% yield by boiling a solution of benzophenone oxime benzenesulphonate (15 g.) and β-naphthol (6·4 g.; 1 mol.) in dry benzene (50 c.c.) for 15 minutes. The salt was decomposed with 2N-sodium hydroxide, and the *N*-phenylbenzimino-β-naphthyl ether (10 g., 70%), crystallised from *iso*propanol or benzene, had m. p. 127° (Found: N, 4·4. Calc. for $C_{23}H_{17}ON$: N, 4·3%). Chapman (*J.*, 1927, 1746) records m. p. 127—128°.

N-*Phenylbenziminoethyl Ether*.—Pyridinium benzenesulphonate (11·4 g.), m. p. and mixed m. p. 130—132°, separated when benzophenone oxime benzenesulphonate (16·3 g.), absolute ethanol (2·2 g.; 1 mol.), dry pyridine (3·8 g.; 1 mol.), and dry benzene (50 c.c.) were boiled for 30 minutes. Distillation of the residue obtained by evaporating the filtrate from this salt afforded *N*-phenylbenziminoethyl ether (10·2 g., 94%), b. p. 120—121°/1 mm. (Found: N, 6·1. Calc. for $C_{15}H_{15}ON$: N, 6·2%). Lander (*J.*, 1902, **81**, 594) records b. p. 168—170°/14 mm.

N-*Benzenesulphonyl*-N'-*phenylbenzamidine*.—When benzophenone oxime benzenesulphonate (15·2 g.) and benzenesulphonamide (7·1 g.) were boiled in benzene solution (50 c.c.) for 30 minutes, 2·35 g. of the sulphonamide were recovered from the cooled reaction product. After being shaken with 2N-sodium hydroxide and kept for several days, the benzene solution deposited benzanilide (3·6 g.), m. p. and mixed m. p. 163°. The alkaline solution was acidified and afforded colourless plates, m. p. 138—140° (4·25 g., 25%), which on crystallisation from 80% aqueous methanol gave pure *N*-benzenesulphonyl-*N'*-phenylbenzamidine, m. p. 140° (Found: N, 8·3. Calc. for $C_{19}H_{16}O_2N_2S$: N, 8·3%). Wallach and Grossmann (*Annalen*, 1880, **214**, 214) record m. p. 138—139°. This compound is soluble in 2N-sodium hydroxide but is insoluble in acids.

N-*Benzoyl*-N'-*phenylbenzamidine*.—(1) A mixture of oil and crystals separated when benzophenone oxime benzenesulphonate (16·35 g.), benzamide (5·9 g.; 1 mol.), and benzene (50 c.c.) were boiled for an hour. Neutralisation of the strongly acid mixture with dilute ammonia caused the oil to dissolve, leaving benzanilide (8·1 g.), m. p. and mixed m. p. 163°. The benzene solution was dried, evaporated, and the mixture afforded phenyl cyanide (4 g., 80%), b. p. 44—45°/1·5 mm., on distillation, leaving a residue (2 g.) which afforded benzanilide (0·3 g.) on trituration with benzene. The filtered benzene solution was extracted with 10N-hydrochloric acid (5 × 5 c.c.), the combined extracts were made alkaline with dilute aqueous ammonia, and the precipitate (0·65 g., 4·5%) of crude *N*-benzoyl-*N'*-phenylbenzamidine, m. p. 137—140°, was collected and crystallised from 50% aqueous methanol to give the pure compound which had the recorded m. p. 143° (Beckmann and Sandel, *Annalen*, 1897, **296**, 286).

(2) When the experiment was repeated in presence of pyridine (7·7 g.; 1 mol.) approximately the same yields were obtained, namely, benzanilide, 89%; phenyl cyanide, 82%; and *N*-benzoyl-*N'*-phenylbenzamidine, 3·8%.

Thermal Decomposition of Oxime Sulphonates.

N-*Benzoylbenzenesulphonanilide*.—A specimen of this compound was prepared in view of Chapman's suggestion that it would be produced by the rearrangement of benzophenone oxime benzenesulphonate. Benzenesulphonanilide (15 g.), benzoyl chloride (9 g.; 1 mol.), and pyridine (10 g.) were heated on the steam-bath for 3 hours, and the product was digested with dilute hydrochloric acid until crystalline. The anilide (18 g.; 77·5%) separated from alcohol in felted needles or in stout prisms, each having m. p. 114°. Freundler (*Bull. Soc. chim.*, 1904, **31**, 624) also observed that this anilide crystallises in needles or in prisms but ascribes different m. p.s, 104° and 114°, respectively, to the two forms. There was no appreciable hydrolysis when the anilide was heated on the steam-bath for 30 minutes with 10% aqueous benzenesulphonic acid.

Benzophenone Oxime Benzenesulphonate.—(1) *NN'*-Diphenylbenzamidinium benzenesulphonate was obtained in 92·5% yield when benzophenone oxime benzenesulphonate and aniline were heated in benzene solution, but if the benzene solution of the oxime ester was boiled before adding the aniline, phenylammonium benzenesulphonate, benzenesulphonanilide, and *N*-benzoyl-*NN'*-diphenylbenzamidine were also formed and became the sole products of the reaction if the addition of aniline was delayed for 15 minutes. A solution of benzophenone oxime benzenesulphonate (16·3 g.) in dry benzene (50 c.c.) was boiled for 15—120 minutes (as indicated in the table below), and aniline (4·5 g.; 1 mol.) was then added to the cooled solution. There was an exothermic reaction, which raised the temperature by 30°, and a crystalline precipitate was formed immediately. The mixture was boiled under reflux for 30 minutes to complete the reaction, and phenylammonium benzenesulphonate, m. p. and mixed m. p. 240—242°,

was collected after cooling. Benzenesulphonanilide, m. p. and mixed m. p. 109°, was extracted from the benzene solution with N-sodium hydroxide and benzoic acid was not detected in the extract. The benzene solution was then extracted with dilute hydrochloric acid to remove aniline, and evaporation of the solvent gave a solid which afforded yellow crystals (8·1 g.), m. p. 160—165°, on trituration with benzene–light petroleum (1 : 1; 50 c.c.). There was a large depression in m. p. when this solid was mixed with benzanilide (m. p. 163°), and crystallisation from benzene afforded pale yellow prisms, m. p. 173°, and a small quantity of a yellow powder, m. p. 145—160°, which were separated mechanically. The powder, which may have contained benzanilide, was not investigated further, and the crystalline product was recrystallised from benzene (charcoal), which gave faintly yellow prisms, m. p. 174·5—175° (Found: C, 83·5; H, 5·4; N, 7·4. Calc. for $C_{26}H_{20}ON_2$: C, 83·0; H, 5·4; N, 7·45%). There was no depression in m. p. when this compound was mixed with *N*-benzoyl-*NN'*-diphenylbenzamidine, m. p. 174·5—175°, prepared in 93% yield from *NN'*-diphenylbenzamidine (2 mols.) and benzoyl chloride in boiling benzene, the hydrochloride of the original amidine separating from the solution. *N*-Benzoyl-*NN'*-diphenylbenzamidine is stated to have m. p. 170—172° (Lander, *J.*, 1902, **81**, 594); 171° (Wheeler and Johnson, *Amer. Chem. J.*, 1903, **30**, 36); or 172° (Mumm, Hesse, and Volquartz, *Ber.*, 1915, **48**, 390) and to yield *NN'*-diphenylbenzamidine and benzoic acid on hydrolysis with hot dilute sulphuric acid (Lander, *loc. cit.*). When this compound (2 g.) and N-benzenesulphonic acid (50 c.c.) were stirred at 100° for 30 minutes the main product was benzanilide. Filtration of the cold product afforded a solid, m. p. *ca.* 125—135° (1·87 g.), which on crystallisation from benzene gave benzanilide (0·85 g.), m. p. and mixed m. p. 161—163°. *NN'*-Diphenylbenzamidine (0·06 g.), m. p. and mixed m. p. 146—147°, was precipitated when 5N-sodium hydroxide was added to the original aqueous filtrate.

When the aniline was added after the benzene solution of the oxime sulphonate had been boiled for 7·5 minutes, the product contained *NN'*-diphenylbenzamidine as well as the transformation products of the imido-sulphonate. The results are summarised in the following table.

Minutes' boiling before adding aniline	0	7·5	15—120
NN'-Diphenylbenzamidinium benzenesulphonate, mols.	0·925	0·23	—
Benzenesulphonanilide, mols.	—	0·35	0·47
Phenylammonium benzenesulphonate, mols.	—	0·36	0·47
N-Benzoyl-*NN'*-diphenylbenzamidine, mols.	—	—	0·5

When *p*-toluidine was used instead of aniline, the product contained benzenesulphon-*p*-toluidide, m. p. and mixed m. p. 121·5° (0·48 mol.), *p*-tolylammonium benzenesulphonate, m. p. and mixed m. p. 206—207° (0·48 mol.), and *N*-benzoyl-*NN'*-diphenylbenzamidine, m. p. and mixed m. p. 173—174°. These results, and the observation that although the benzene solution of the oxime ester contained no free benzenesulphonic acid after 15 minutes' boiling, it gave an immediate precipitate of phenylammonium benzenesulphonate on adding aniline, suggested that the solution contained benzenesulphonic anhydride. (2) A solution of benzophenone oxime benzenesulphonate (15 g.) in dry benzene (45 c.c.) which had been boiled for 30 minutes, was cooled, diluted with light petroleum (30 c.c.; b. p. 60—80°), and kept in the refrigerator overnight. The crystals (7·0 g., 84%) which separated had m. p. 168—171°, and crystallisation from benzene gave *N*-benzoyl-*NN'*-diphenylbenzamidine, m. p. and mixed m. p. 174—175°. The reddish-brown gum (8 g.) obtained by evaporating the original filtrate afforded benzenesulphonic anhydride (4·9 g., 74%), m. p. 87—90°, when extracted exhaustively with boiling ligroin (b. p. 80—100°), and recrystallisation from benzene–light petroleum raised the m. p. to 91—92° in agreement with the literature value (Billeter, *Ber.*, 1905, **38**, 2016; Meyer and Schlegl, *Monatsh.*, 1913, **34**, 569). The anhydride (2·98 g.) and aniline (1·9 g.) in benzene solution gave phenylammonium benzenesulphonate (2·49 g., 99%), m. p. and mixed m. p. 240—242°, and benzenesulphonanilide (2·28 g., 98%), m. p. and mixed m. p. 109°. (3) There was an exothermic reaction when benzophenone oxime benzenesulphonate (10 g.), benzene (30 c.c.), and dry pyridine (2·35 g.; 1 mol.) were warmed on the steam-bath. A heavy brown oil was rapidly deposited and, after an additional 30 minutes' boiling, aniline (2·8 g., 1 mol.) was added and the exothermic reaction was completed by boiling for 15 minutes. The reddish crystals (11 g.), m. p. 190—205°, which separated on cooling were recrystallised from *iso*propanol (50 c.c.) and afforded *N*-benzoyl-*NN'*-diphenylbenzamidinium benzenesulphonate, m. p. and mixed m. p. 217—218° (8·95 g., 70%). The same yield of this compound was obtained when a solution of the oxime ester (10 g.) in dry pyridine (20 c.c.) was heated to 100° for 30 minutes before addition of aniline, and it is clear that pyridine inhibits the decomposition of the imidosulphonate produced by rearrangement of the oxime benzenesulphonate.

*Benzophenone Oxime Toluene-*p*-sulphonate.*—(1) A solution of benzophenone oxime toluene-*p*-sulphonate (16 g.) in dry benzene (50 c.c.) was boiled for an hour, aniline (4·5 g.; 1 mol.) was then added, and boiling continued for 30 minutes. Phenylammonium toluene-*p*-sulphonate (5·75 g., 47·6%), m. p. and mixed m. p. 241°, was collected from the cooled product. Dermer and Dermer (*J. Org. Chem.*, 1942, **7**, 581) state that this salt has m. p. 238°. Toluene-*p*-sulphonanilide (5·34 g., 47·4%), m. p. and mixed m. p. 103°, was extracted from the benzene solution by aqueous sodium hydroxide, and evaporation of the solvent afforded crude *N*-benzoyl-*NN'*-diphenylbenzamidine (8·6 g.), m. p. 155—167°, which gave the pure compound, m. p. and mixed m. p. 173—174°, on recrystallisation from benzene. (2) Benzophenone oxime toluene-*p*-sulphonate (16·2 g.) and benzene (50 c.c.) were boiled for 30 minutes; the solution was evaporated to dryness and the residue was triturated with anhydrous ether (3 × 25 c.c.). The residue afforded *N*-benzoyl-*NN'*-diphenylbenzamidine (7·1 g., 85·5%), m. p. and mixed m. p. 174—175°, on crystallisation from benzene. The ethereal solution was evaporated, and the residue crystallised from benzene–light petroleum (1 : 1), giving toluene-*p*-sulphonic anhydride, m. p. 122—124° (4·5 g., 60%). Meyer and Schlegl (*loc. cit.*) record m. p. 123—125°. The anhydride was characterized further by the production of phenylammonium toluene-*p*-sulphonate and toluene-*p*-sulphonanilide in almost quantitative yield by reaction with aniline in benzene solution.

Acetophenone Oxime Benzenesulphonate.—(1) A heavy dark oil separated when a solution of the oxime ester (23 g.) in dry benzene (70 c.c.) was boiled for 30 minutes. When aniline (7·8 g.; 1 mol.) was added

to the cold reaction product, the oil dissolved and phenylammonium benzenesulphonate (5·5 g., 26·1%), m. p. and mixed m. p. 238—240°, separated. Benzenesulphonanilide (3·43 g., 17·6%), m. p. and mixed m. p. 109°, was extracted from the benzene solution by 2N-sodium hydroxide, and subsequent extraction with N-hydrochloric acid removed the basic products and left only 2·2 g. of a brown oil, having a strong odour of acetophenone, in the benzene solution. A pasty brown solid (10 g.) was liberated when the acid extract was made alkaline with sodium hydroxide, and crystallisation from *iso*propanol (25 c.c.) afforded *NN'*-diphenylacetamidine (5·7 g.), m. p. and mixed m. p. 132·5°. The *iso*propanol solution contained aniline. (2) There was an exothermic reaction lasting for 5 minutes when a solution of the oxime ester (69 g.) in dry benzene (200 c.c.) was warmed, and a dark oil separated from the solution. After being boiled for 30 minutes, the mixture was cooled and shaken with water. The strongly acid aqueous layer required *ca.* 0·33 mol. of potassium hydroxide (per mol. of oxime ester) to neutralise it to Congo-red, and a further 0·05 mol. to neutralise it to brilliant-yellow. The oil had dissolved and the benzene layer was separated, extracted with dilute hydrochloric acid, dried, and evaporated, giving a dark brown oil (24·2 g.) which was distilled. There was a small forerun (1·3 g.), b. p. up to 100°/1 mm., smelling strongly of acetophenone, a fraction (9 g., 27%), b. p. 130—140°/0·5 mm., which proved to be acetanilide, m. p. and mixed m. p. 114°, and an intractable residue which could not be distilled without decomposition. The hydrochloric acid extract was made alkaline with 5N-sodium hydroxide and extracted with chloroform to give a brown oil (7·85 g.) which afforded a solid hydrochloride. Crystallisation from *iso*propanol gave *NN'*-diphenylacetamidinium chloride (5 g., 16%), m. p. and mixed m. p. 224°. In another experiment it was found that when the initial reaction product was neutralised with alkali and then digested with water a further 0·16 mol. of potassium hydroxide was required to render the aqueous layer neutral to Congo-red and 0·27 mol. to make it neutral to brilliant-yellow. This result suggests that the product contained benzenesulphonic anhydride. Aniline (1·9 g.), identified as phenylammonium benzenesulphonate, m. p. and mixed m. p. 241°, was also isolated from the basic products in this experiment.

p-*Bromoacetophenone Oxime Benzenesulphonate.*—A solution of the oxime ester (16·7 g.) in benzene (30 c.c.) was boiled for 30 minutes, and, after this had been cooled, *p*-bromoaniline (8·1 g.; 1 mol.) was added. p-*Bromophenylammonium benzenesulphonate* (3·27 g., 27%) was precipitated immediately and after crystallisation from methanol had m. p. 238°, undepressed on admixture with an authentic sample prepared from the constituents in *iso*propanol (Found: N, 4·4. $C_{12}H_{12}O_3NBrS$ requires N, 4·2%). The filtrate from the aniline salt slowly deposited *NN'*-bis-*p*-bromophenylacetamidinium benzenesulphonate (13·75 g., 55·4%), m. p. and mixed m. p. 162—165°. The benzene solution was finally extracted with warm dilute sodium hydroxide, and the alkaline solution was treated with charcoal and acidified, giving benzenesulphon-*p*-bromoanilide (2·39 g., 16%), m. p. 133—134°, undepressed on admixture with an authentic specimen, m. p. 135°, prepared as described by v. Braun (*Ber.*, 1907, **40**, 3926), who records m. p. 134°.

Acetoxime Benzenesulphonate.—(1) *Decomposition in toluene.* This oxime ester did not undergo rearrangement on boiling a solution in benzene or in dichloroethylene, but there was a slow reaction in boiling toluene. A brown oil separated when a solution of the oxime ester (21·3 g.) in dry toluene (50 c.c.) was heated and, although the same products were ultimately obtained by either method, direct heating over a gauze was preferred, since heating in an oil-bath resulted in quiet boiling for a few minutes followed by a violent reaction and separation of the oil. The yield of oil insoluble in toluene was 7·0, 10·5, 17·95, and 19·85 g. after heating for 0·5, 0·75, 2·0 and 3·0 hours, respectively. Unless the solution was boiled for 3 hours, unchanged oxime ester was still present and rearranged violently when the solution was concentrated. Except where otherwise indicated, it is to be assumed in the following description that the toluene solution had been boiled for 3 hours. The toluene solution and the insoluble oil were separated and examined.

(*a*) The toluene solution afforded an almost colourless gum on evaporation, and on being scratched with a glass rod gave crystals, m. p. *ca.* 85° (1·3 g., 8·7%). Several crystallisations from benzene–light petroleum (1 : 2) gave colourless, flat needles of benzenesulphonic anhydride, m. p. 91—92° (Found: C, 47·6; H, 3·3. Calc. for $C_{12}H_{10}O_5S_2$: C, 48·3; H, 3·4%). In other experiments, excess of aniline was added to the toluene solution, the precipitated phenylammonium benzenesulphonate (1·06 g.), m. p. and mixed m. p. 240—241°, was collected, and benzenesulphonanilide (0·98 g.), m. p. and mixed m. p. 109°, was extracted from the filtrate with dilute aqueous sodium hydroxide. When the toluene solution of the oxime ester was boiled for ½ hour the yields of aniline salt and anilide were 1·27 and 1·16 g., respectively. The toluene solution remaining after the removal of the benzenesulphonanilide was freed from aniline by washing with dilute hydrochloric acid, dried, and added to the similar solutions obtained in 4 other experiments (each with 21·3 g. of oxime ester). The toluene was removed, and the residual oil (5·2 g.) distilled under diminished pressure. The fraction, b. p. 109°/1·5 mm., or *ca.* 275°/760 mm. (4·5 g.), consisted of methyl benzenesulphonate (Found: C, 48·3; H, 4·5. Calc. for $C_7H_8O_3S$: C, 48·8; H, 4·65%), which was characterized by reaction with aniline. A solution of the ester (1·4 g.) and aniline (1·5 g.; 2 mols.) in benzene (10 c.c.) was boiled under reflux for 45 minutes, cooled, and filtered to yield phenylammonium benzenesulphonate, m. p. and mixed m. p. 235—240° (1·9 g., 95%). The benzene solution was extracted with dilute hydrochloric acid, and the basic oil (0·67 g.) liberated on adding sodium hydroxide to the acid solution was added to a methanolic solution of picric acid (1·5 g.). The precipitate was collected (0·6 g., m. p. 152—158°) and afforded phenyldimethylammonium picrate, m. p. and mixed m. p. 162°, on crystallisation from methanol. The filtrate was evaporated to dryness and the residue crystallised from *iso*propanol, giving phenylmethylammonium picrate (0·6 g.), m. p. 140—145°, raised to 144—145° by a second crystallisation and undepressed on admixture with an authentic specimen.

(*b*) The oil insoluble in toluene dissolved in water to a solution which consistently required 8·7 ml. of 5N-potassium hydroxide for adjustment to pH 3·5 (bromophenol-blue) and an additional 8·5—9·0 ml. for adjustment to pH 12 (Clayton-yellow), suggesting the presence of approximately equal quantities of benzenesulphonic acid in the free condition and in combination with bases. The oil was dissolved in water (20 c.c.), adjusted to pH 3·5 with 5N-potassium hydroxide, and filtered through a column of charcoal (20 g.). The column was eluted successively with water (100 c.c.), methanol (150 c.c.), and chloroform

(100 c.c.), each operation being carried to exhaustion. The chloroform solution contained only 0·2 g. of a dark brown gum, readily soluble in water to a weakly acid solution (pH 5), and was not investigated further. Evaporation of the methanolic eluate afforded an orange-brown gum (*ca.* 1·8 g.) which was dissolved in water (5 c.c.) and mixed with 2M-lithium picrate (5 c.c.), and the sticky precipitate collected. Recrystallisation from M-lithium picrate (20 c.c.) removed a gum and afforded orange crystals of a picrate, m. p. 155—160°, the yield varying from 0·52 to 0·61 g. Successive crystallisation from methanol (50 parts) afforded the pure *picrate*, m. p. 166° (Found: C, 44·3, 44·0; H, 4·4, 4·2; N, 22·0, 22·1. $C_{14}H_{16}O_7N_6$ requires C, 44·2; H, 4·2; N, 22·1%). This is the picrate of the base $C_8H_{12}N_2$ and the further investigation of this compound and its derivatives is described below. The aqueous eluate, which smelled of acetic acid, was evaporated to dryness, and the residue of sticky crystals was separated into portions soluble and insoluble in acetone (100 c.c.). The insoluble portion (9·45 g.) was separated by *iso*propanol into potassium benzenesulphonate (8·5 g.) (Found: C, 36·9; H, 2·85. Calc. for $C_6H_5O_3SK$: C, 36·7; H, 2·6%) and a soluble portion which gave methylammonium benzenesulphonate, m. p. and mixed m. p. 166° (0·51 g.), after a further crystallisation from *iso*propanol. The portion of the aqueous eluate soluble in acetone consisted of an orange gum (5·9 g.) which was dissolved in water (5 c.c.) and mixed with 2M-lithium pircate (12·5 c.c.). The precipitate (3·62 g., m. p. 128—130·5°) afforded *NN'*-dimethylacetamidinium picrate, m. p. and mixed m. p. 132—132·5°, on crystallisation from *iso*propanol.

(2) *Decomposition in other solvents.* Acetoxime benzenesulphonate (21·3 g.) afforded the same products when boiled in solution in xylene (50 c.c.) and these were separated as described in (1). After 15 minutes' boiling, the xylene-soluble products afforded phenylammonium benzenesulphonate (1·3 g.) and benzenesulphonanilide (1·14 g.) on reaction with aniline. The yield of the picrate of the base $C_8H_{12}N_2$ was higher (1·23 g., m. p. *ca.* 160°) than in toluene solution but the other products were obtained in similar yield. A high yield (1·25 g., m. p. 160—163°) of this picrate was also obtained in the same way when the oxime ester was boiled in *p*-cymene solution for 5 minutes, but the yield fell to 0·51 g. when decomposition of the oxime ester was effected at 180° for 30 minutes in nitrobenzene solution.

(3) *Decomposition at room temperature.* Acetoxime benzenesulphonate could usually be kept unchanged at room temperature for several weeks, but one sample (120 g.) decomposed within 3 months to give a mixture of crystals and yellow gum having a strongly acid reaction. Trituration with *iso*propanol (120 c.c.) dissolved most of the mixture, leaving a crystalline residue (8·3 g., m. p. 135—141°), which appeared to consist of a mixture of ammonium and methylammonium benzenesulphonate. The residue obtained by evaporating the *iso*propanol solution was dissolved in water (100 c.c.), brought to pH 4·0 (Congo-red) by adding 5·4N-potassium hydroxide (48 ml.), and filtered through charcoal (50 g.). The charcoal was extracted with water (400 c.c.) and then with methanol (500 c.c.). The methanolic solution was evaporated to dryness and a solution of the residue in water (5 c.c.) was added to 2M-lithium picrate (25 c.c.). After being kept for 2 days, the precipitate was collected (1·06 g., m. p. 115—150°) and crystallised from methanol and methanol–acetone to give a *picrate* (0·25 g.), separating in flat needles, m. p. 231° (Found: C, 45·4; H, 4·4; N, 21·4. $C_{15}H_{18}O_7N_6$ requires C, 45·7; H, 4·6; N, 21·3%). This may be a methopicrate corresponding to the methobenzenesulphonate formulated on p. 1517.

Investigation of the Base $C_8H_{12}N_2$.—The picrate, m. p. 166° (3·8 g.), was shaken with N-toluene-*p*-sulphonic acid (10 c.c.) and 2N-hydrochloric acid (10 c.c.) and the mixture was then extracted with benzene. The residue obtained by concentrating the aqueous solution was recrystallised from *iso*propanol, giving buff crystals of the *toluene*-p-*sulphonate*, m. p. 166—166·5° (Found: C, 56·1; H, 6·5; N, 13·0. $C_{15}H_{21}O_3N_3S$ requires C, 55·7; H, 6·5; N, 13·0%). The picrate (2·3 g.), 2N-hydrochloric acid (25 c.c.), and benzene (25 c.c.) were shaken until solution was complete, and the aqueous solution was then extracted twice with benzene and evaporated to dryness. The resulting green crystals (1·2 g., m. p. *ca.* 220—222°, decomp.) were crystallised from *iso*propanol and dried over phosphoric oxide at 100°/1 mm., giving the deliquescent *hydrochloride*, m. p. 222—223° (decomp.) (Found: C, 49·0; H, 7·1; N, 23·0. $C_8H_{14}N_2Cl$ requires C, 51·2; H, 7·5; N, 22·4%). The base was liberated from these salts by sodium hydroxide as a sticky solid which could not be purified owing to the ease with which it is oxidised in air with production of a pink colour.

There was no appreciable hydrolysis when the salts were boiled with 2N-hydrochloric acid or with aqueous sodium carbonate but hydrolysis occurred with boiling aqueous sodium hydroxide. (1) The aforementioned picrate (1·90 g.) was heated with 1·9N-sodium hydroxide (40 c.c.), water being added at intervals to replace that which had distilled. Evolution of basic vapours was complete when the distillate amounted to 100 c.c., and 16·2 ml. of N-toluene-*p*-sulphonic acid (equivalent to 3·2 mols. per mol. of $C_8H_{12}N_2$) were required to neutralise the distillate to methyl-orange. The neutral solution was evaporated to dryness and the residue was extracted with *iso*propanol, leaving undissolved ammonium toluene-*p*-sulphonate, m. p. and mixed m. p. 345—346° (0·82 g.; 0·87 mol. per mol. of $C_8H_{12}N_2$). Evaporation of the *iso*propanol and trituration of the residue with acetone afforded slightly impure methylammonium toluene-*p*-sulphonate, m. p. 140—144° (1·96 g.; 1·93 mol. per mol. of $C_8H_{12}N_2$), and crystallisation from *iso*propanol gave the pure salt, m. p. 146—147°, undepressed on admixture with a specimen, m. p. 147°, prepared from its constituents in *iso*propanol (Found: N, 6·8. Calc. for $C_8H_{13}O_3NS$: N, 6·9%). Norton and Otten (*Amer. Chem. J.*, 1888, **10**, 140) state that this salt has m. p. 125°. (In a series of model experiments it was found that boiling *iso*propanol or methyl ethyl ketone was the most suitable solvent for the separation of a 1 : 2 mixture of ammonium toluene-*p*-sulphonate and methylammonium toluene-*p*-sulphonate.) (2) The toluene-*p*-sulphonate (1·62 g.) was distilled with 5N-sodium hydroxide (20 c.c.), the distillate being collected in N-toluene-*p*-sulphonic acid (15 c.c.). Water was added to the residue at intervals and distillation was continued until the distillate was neutral to methyl-orange (2 hours).

(*a*) The distillate was redistilled and the first 5 c.c. was mixed with a 2% solution of *p*-nitrophenylhydrazine in 30% acetic acid (5 c.c.). The precipitate so obtained (0·10 g.; *ca.* 0·1 mol.) had m. p. 148—149° (Found: N, 21·8. Calc. for $C_9H_{11}O_2N_3$: N, 21·75%), undepressed on admixture with an authentic specimen of acetone *p*-nitrophenylhydrazone, m. p. 149°. The second 5 c.c. of distillate gave only an opalescence with the reagent.

(*b*) The residue was acidified with concentrated sulphuric acid (8 c.c.) and distilled so long as an acid

distillate was obtained (50 c.c.; ½ hour). Titration of the distillate with N-sodium hydroxide (13·5 ml.), using phenolphthalein, indicated the presence of 2·7 mols. of a monobasic acid (per mol. of $C_5H_{13}N_3$). The neutral solution was evaporated and the residue was dehydrated by adding *iso*propanol and distilling to dryness. The residual white crystals (1·1 g.; 2·7 mols.) consisted of anhydrous sodium acetate, m. p. and mixed m. p. 332—333°, further identified by conversion into *p*-nitrobenzyl acetate, m. p. and mixed m. p. 78°.

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306. *The Constitution of Yeast Ribonucleic Acid. Part XII. Synthesis of Cytidine-2′ Phosphate.*

By (the late) J. MASSON GULLAND and H. SMITH.

Cytidine-2′ phosphate has been synthesised from benzylidene cytidine by procedures which follow the general pattern of the synthesis of uridine-2′ phosphate previously described; phosphorylation was, however, effected by means of phosphorus oxychloride instead of diphenyl chlorophosphonate. The reagent attacked both amino- and sugar hydroxyl groups, the former preferentially, thus necessitating selective fission of the phospho-amide linkages. The stabilities of cytidine-2′ phosphate and cytidylic acid toward alkali are almost identical, and this confirms the view previously expressed that the facile alkaline fission of yeast ribonucleic acid is not due to a marked and inherent lability of the phospho-ester linkage in position 2′, but is a property of the polynucleotide.

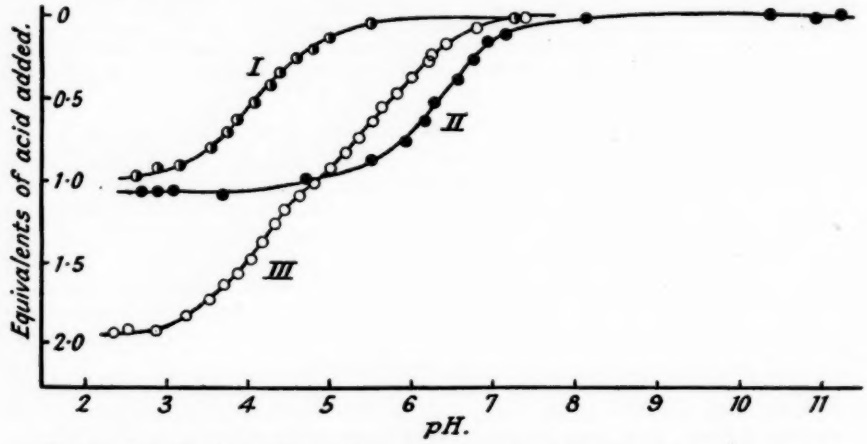
IN a previous paper (Gulland and Smith, *J.*, 1947, 338) we reported the synthesis of uridine-2′ phosphate, and from its stability toward alkali concluded that there is no inherently greater lability of a phosphoryl group in the 2′, as compared with the 3′, position, and that the problem of the positions of the internucleotide linkages of yeast nucleic acid was thus reopened. We now describe the synthesis of *cytidine-2′ phosphate*, and a study of its properties reveals so close a parallelism with those of uridine-2′ phosphate that our previous conclusions receive further strong support. The general scheme of this synthesis followed the earlier work in that the 3′ and 5′ hydroxyl groups of the sugar were blocked by the benzylidene radical during phosphorylation of the 2′ position, but the presence of the amino-group introduced complications which necessitated a modification of the phosphorylation technique.

Beautifully crystalline cytidine was obtained by dephosphorylation of cytidylic acid with boiling aqueous pyridine. Its m. p. 211—214° (decomp.), unchanged by repeated recrystallisation, differed from that recorded by Levene and La Forge (*Ber.*, 1912, **45**, 608), namely sintering at 220° and decomposition at 230°; we have found no other record of the m. p. of cytidine for comparison, but the figure given above has been obtained repeatedly with different preparations. The two compounds were undoubtedly identical, since the specific rotation of our material was the same as that given by Levene and La Forge, and the m. p. of the sulphate and picrate agreed with those recorded by other workers (Levene and Jacobs, *Ber.*, 1910, **43**, 3150; Davoll, Lythgoe, and Todd, *J.*, 1946, 833).

Interaction of cytidine, benzaldehyde, and zinc chloride yielded a zinc-containing complex which could not be fractionated, but dry hydrogen chloride proved an excellent condensing agent, and a good yield of 3′ : 5′-*benzylidene cytidine* (I) was obtained. It was demonstrated that no change had occurred in the furanose ring structure of the sugar by the recovery of cytidine after hydrolysis of the benzylidene derivative with dilute acid. The titration curve of benzylidene cytidine (figure, curve I) was almost identical with that of cytidine, the pK' of the amino-group being 4·1 for the benzylidene derivative as compared with 4·2 for cytidine (Levene and Simms, *J. Biol. Chem.*, 1925, **65**, 519), and this afforded proof that the benzaldehyde had condensed with the sugar radical and not with the amino-group in the form of a Schiff's base. Determination of the position of the benzylidene group on the sugar presented difficulties. Since cytidine-5′ phosphate has not been described, the method of comparing the properties of the 2′-, 3′-, and 5′-phosphates which proved the structure of 3′ : 5′-benzylidene uridine (Gulland and Smith, *loc. cit.*) could not be applied here. For the present, therefore, knowledge of the structure of 3′ : 5′-benzylidene cytidine, and hence of the position of the phosphoryl in cytidine 2′-phosphate, rests on the analogy with the benzylidene derivatives of uridine and of guanosine (Gulland and Overend, this vol., p. 1380), on the experimentally demonstrated difference between cytidine-2′ phosphate and cytidylic acid (see below), and on the parallel behaviour of the cytidine and uridine phosphates towards alkali and acid.

The first phosphorylating agent to be examined was diphenyl chlorophosphonate, and analysis of the product by the action of one molecular proportion of this on benzylidene cytidine in dry pyridine at — 20° showed that it was a mixture of benzylidene cytidine diphenyl phosphate and a smaller quantity of benzylidene cytidine tetraphenyl diphosphate. Two properties revealed that phosphorylation had occurred preferentially at the amino-group and that the major constituent of the mixture was the diphenyl-N^6 phosphate. First, the material was insoluble in dilute mineral acid. Secondly, investigation of the behaviour of this product during attempts to remove the phenyl residues and convert it into cytidine-2′ phosphate showed that the bulk of the phosphoryl radical was converted into inorganic phosphate by mild acid or alkaline hydrolysis followed by hydrogenolysis of the surviving phenyl radical. This change could not have occurred if the phosphoryl radical had esterified a sugar hydroxyl, as will be clear from a study of the method of preparation and properties of uridine-2′ phosphate (Gulland and Smith, *loc. cit.*) and of cytidine-2′ phosphate (see below).

Attention was then turned to double phosphorylation, to the preparation of *3′ : 5′-benzylidene cytidine tetraphenyl-*N^6 *: 2′ diphosphate* (II) from which the benzylidene radical and *N*-phosphoryl might be removed simultaneously by acid hydrolysis, and in which hydrogenolysis might then remove the phenyl radicals from the phosphoryl remaining in the 2′-position. Condensation of



Electrometric titrations of benzylidene cytidine (I), *the deaminated nucleotide* (II), *and cytidine-2′ phosphate* (III).

benzylidene cytidine with 2·2 mols. of diphenyl chlorophosphonate yielded the compound (II), thus confirming that phosphorylation of both the amino- and the hydroxyl group occurred and providing a parallel with the work of Levene and Tipson (*J. Biol. Chem.*, 1937, **121**, 131) on the tritylation, tosylation, and phosphorylation of adenosine or its derivatives. The compound (II) was hydrolysed with hot N/4-hydrochloric acid in methyl alcohol, and the resulting product hydrogenated at room temperature and atmospheric pressure in presence of Adams's platinum catalyst. Free ammonia was detected in the solution, from which was isolated the barium salt of a deaminated nucleotide, possibly 2-keto-2 : 3-dihydropyrimidine-3-(ribofuranosido-2′ phosphate).

The nature of the nitrogenous ring structure of this compound has not yet been fully elucidated, but the absence of an amino-group was clearly indicated by analysis and confirmed by electrometric titration (figure, curve II), in which the only dissociation group between pH3 and 11 was that of a secondary phosphoryl. It was shown that preferential hydrogenolysis of the phenyl radicals without removal of the amino-group was not possible under the conditions used; thus, hydrogenation of an equimolecular mixture of phenyl phosphate and cytidylic acid caused the absorption of that volume of hydrogen theoretically required for one phenyl group, but only half of the phenyl phosphate had been converted into inorganic phosphate whereas the theoretical quantity of ammonia corresponding to the amino-group was present.

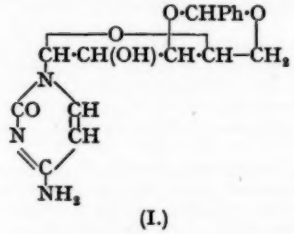
The conditions and mechanism of the aminolysis are not clear. Although Levene and Jorpes (*ibid.*, 1929, **81**, 575) did not observe the liberation of ammonia when cytidylic acid was hydrogenated using Adams's platinum catalyst, we obtained a quantitative yield of ammonia

by hydrogenating cytidylic acid at room temperature for 1½ hours in hydrogen at one atmosphere pressure. Brown and Johnson (*J. Amer. Chem. Soc.*, 1924, **46**, 702) hydrogenated cytosine at 75° for 7 hours at a pressure of 2 atmospheres in presence of platinum, and the product readily split in the acid medium into dihydrouracil and ammonia. In the present case the product of a similar reaction would contain an enolic hydroxyl group similar in its dissociation constant to that of dihydrouracil which has pK' 11·5 (Levene, Bass, and Simms, *J. Biol. Chem.*, 1926, **70**, 229); the figure, curve II, shows, however, that this group was absent.

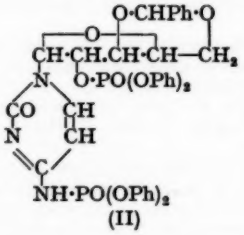
It thus became evident that in this synthesis a method of phosphorylation which did not involve hydrogenolysis would be preferable, and attention was turned to the use of phosphorus oxychloride. Attempts to protect the amino-group in the form of a Schiff's base with benzaldehyde and with *p*-nitrobenzaldehyde were unsuccessful, but better prospects were offered by simultaneous phosphorylation of the amino- and sugar hydroxyl groups followed by preferential hydrolysis of the *N*-phosphoryl radical; Levene and Tipson (*loc. cit.*) had limited success with a similar procedure in the synthesis of adenosine-5′ phosphate.

Benzylidene cytidine was phosphorylated in dry pyridine at — 20° in presence of 3·2 mols. of phosphorus oxychloride, the formation of a derivative of di- or possibly even tri-cytidine monophosphate being avoided by addition of benzylidene cytidine in small portions to the oxychloride, so as to ensure that the latter was constantly present in excess. After hydrolysis of the contents of the reaction mixture with cold water, determination of the amount of uncombined phosphoric acid showed that 3 mols. of phosphorus oxychloride had reacted. It is considered that the primary product was 3′ : 5′-benzylidene cytidine 2′ : N^6 : N^6-trisdichlorophosphinate or the corresponding N^1 : N^6-derivative. Hydrolysis of the product with N/4-hydrochloric acid at 100° for 2 hours removed the benzylidene radical and set free rather more than the theoretical quantity of phosphoric acid to correspond with two *N*-phosphoryl groups. After being isolated as the lead salt, cytidine-2′ phosphate (III) was obtained in reasonably good yield in the form of its crystalline *barium* salt, from which the crystalline nucleotide was prepared. Electrometric titration (figure, curve III) showed the presence of an amino-acid and a secondary phosphoryl group, thus proving that this compound was not a phosphoamide and that preferential hydrolysis of both *N*-phosphoryl groups had occurred. Comparison of the crystalline forms, m. p., acid-hydrolysis curves, and specific rotations of the nucleotides and of the rotations of their barium salts revealed clearly that synthetic cytidine-2′ phosphate and cytidylic acid are different, and since preferential participation by the 5′-hydroxyl in the benzylidenation and its consequent blocking seem almost certain these differences provide strong support that the synthetic nucleotide is the 2′-phospho-ester, since the long-held assumption that cytidylic acid is the 3′-derivative has now been proved (Gulland and Smith, *loc. cit.*).

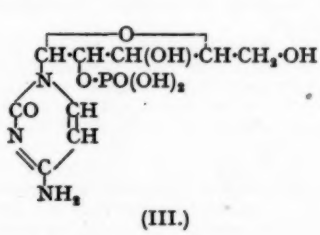
Cytidine-2′ phosphate resembled uridine-2′ phosphate in being completely stable to cold 1% sodium hydroxide for 3 days, conditions which decompose yeast ribonucleic acid into nucleotides. Comparison of the hydrolysis curves in hot acid and alkali shows a strong analogy between cytidine-2′ and -3′ phosphates and the corresponding uridine compounds; the -2′ phosphate is more stable to acid than is the -3′ phosphate, and the stabilities in alkali are closely similar, the -2′ phosphate being slightly the more labile, but the difference is far too minute to justify any suggestion of complete fission of the 2′ phosphoryl linkage in preference to the 3′ linkage (for the significance of this observation, see Gulland and Smith, *loc. cit.*).



(I.)



(II)



(III.)

Experimental.

Cytidine.—A solution of cytidylic acid, m. p. 228—232° (36 g.), in 50% aqueous pyridine (1600 c.c.) was refluxed for 51 hours, by which time about 95% of the total phosphorus had been converted into inorganic phosphate. Pyridine was removed by repeated evaporation under reduced pressure after additions of water. Addition of hot barium hydroxide solution until alkalinity to phenolphthalein was reached and filtration from barium phosphate removed inorganic phosphate. After concentration of the filtrate and washings, excess of barium hydroxide was removed by passing carbon dioxide, boiling, and

filtration. The filtrate and washings (400 c.c.) were mixed with absolute industrial alcohol (1600 c.c.), and the small amount of precipitated barium cytidylate was filtered off and washed with 80% alcohol. After concentration of the filtrate and washings and repeated evaporation with water to remove alcohol, N-sulphuric acid (164 c.c., equivalent to 95% of the cytidylic acid used) was added, and the solution was concentrated to 200 c.c., filtered through charcoal, brought to boiling point, and boiling absolute industrial alcohol (about 800 c.c.) added until there were signs of precipitation. Cytidine sulphate (22·0 g.) separated in long needles from the solution as it cooled, and was recrystallised by solution in boiling water (180 c.c.) and addition of boiling absolute industrial alcohol (700 c.c.).

Cytidine sulphate, m. p. 230—233° (22 g.), was dissolved in water (1 l.) and sulphate ions precipitated by a slight excess of barium hydroxide. After filtration, washing, and concentration of the combined filtrate and washings, excess of barium hydroxide was removed by passage of carbon dioxide, boiling, and filtration. The filtrate and washings were concentrated (60 c.c.), brought to boiling point, and boiling absolute industrial alcohol (540 c.c.) added. On cooling, cytidine (17 g.) crystallised; it recrystallised from 90% alcohol (35 pts.) in long needles, m. p. 211—214° unchanged by repeated recrystallisation, $[\alpha]_D^{17°}$ + 29·7° (*c*, 9·1) in water (Found: C, 44·3; H, 5·3; N, 17·2. Calc. for $C_9H_{13}O_5N_3$: C, 44·4; H, 5·4; N, 17·3%). The picrate separated from 95% alcohol as yellow crystals, m. p. 183—186°, after sintering at 180°. Levene and La Forge (*loc. cit.*) give the optical rotation of cytidine as $[\alpha]_D^{21°}$ + 29·63°; Levene and Jacobs (*loc. cit.*) give the m. p. of the picrate as 185—187° and of the sulphate as 233°, and Davoll, Lythgoe, and Todd (*loc. cit.*) give the m. p. of the picrate as 183°.

3′ : 5′-*Benzylidene Cytidine.*—Cytidine (8 g.) dried at 100° over phosphoric oxide in a vacuum for 4 hours, was suspended in freshly distilled benzaldehyde (80 c.c.) protected from atmospheric moisture. A rapid stream of dry hydrogen chloride was passed through the stirred suspension for 3 hours; the thick white suspension which formed at first became much less viscous at the end of the condensation. The suspension was poured into ether (800 c.c.) and washed thoroughly with saturated sodium carbonate solution (about 80 c.c.) followed by successive small quantities of water. Crude *benzylidene cytidine* (8·1 g.) was collected, washed with ether, and recrystallised twice from water (10 parts) from which it (6·8 g.) separated in colourless needles, m. p. 193—195° (Found: C, 57·8; H, 5·2; N, 12·5. $C_{16}H_{17}O_5N_3$ requires C, 58·0; H, 5·2; N, 12·7%).

Cytidine was regenerated by hydrolysis of benzylidene cytidine (1 g.) at 100° for 1 hour with N/4-sulphuric acid (100 c.c.). After removal of the benzaldehyde by extraction with ether and of the sulphuric acid by means of barium hydroxide, the solution was evaporated to a syrup which was dissolved in hot absolute alcohol (10 c.c.). On cooling, cytidine (0·6 g.) separated in long colourless needles and was recrystallised from 90% alcohol; m. p. 211—214° alone or mixed with an authentic sample; $[\alpha]_D^{15°}$ + 29·4° (*c*, 10·5).

Conversion of Benzylidene Cytidine into a Mixture of Benzylidene Cytidine Diphenyl-N⁴ Phosphate and Tetraphenyl-N⁴ : 2′ Diphosphate.—A solution of freshly distilled diphenyl chlorophosphonate (0·63 c.c.) (Brigl and Müller, *Ber.*, 1939, **72**, 2121) in dry pyridine (10 c.c.) was added during 15 minutes to a mechanically stirred solution of dry benzylidene cytidine (1 g.) in dry pyridine (20 c.c.), cooled in an ice–salt mixture and protected from moisture; stirring was continued for 2 hours in the freezing mixture and then for 1 hour at room temperature. The faintly yellow solution was again cooled in the freezing mixture, 50% aqueous pyridine (5 c.c.) was added during 15 minutes, and stirring was continued for 1 hour. The solution was poured into ice-water (200 c.c.), and the pyridine and water removed under reduced pressure, the volume being reduced to 10 c.c. After 2 successive additions of alcohol followed by evaporation under reduced pressure, the solution (30 c.c.) was cooled in a freezing mixture and poured very slowly into ice–water (100 c.c.). After further standing in the refrigerator, the colloidal suspension was flocculated by addition of saturated sodium chloride solution (2 c.c.). The solid (1·2 g.) was collected, washed with ice–water, dried in a vacuum desiccator over phosphoric oxide, dissolved in alcohol (30 c.c.), and reprecipitated as described above. The product (1·1 g.) was a white amorphous powder, insoluble in water and dilute acid (Found: N, 7·1, 7·1; P, 5·7, 5·8. Calc. for $C_{28}H_{26}O_8N_3P$: N, 7·5; P, 5·5. $C_{40}H_{35}O_{11}N_3P_2$ requires N, 5·3; P, 7·8%).

This material (0·5 g., equivalent to 28 mg. of phosphorus) was hydrolysed with hot N/2-alcoholic sodium hydroxide for 1 hour and hot N/2-alcoholic hydrochloric acid for a further 1 hour in order to remove one phenyl residue and the benzylidene residues, and the remaining phenyl radical was removed by hydrogenolysis in presence of Adams's catalyst. The bulk of the phosphorus (23 mg.) was then present as inorganic phosphate. A similar result was obtained when the preliminary alkaline hydrolysis was omitted.

3′ : 5′-*Benzylidene Cytidine Tetraphenyl-N⁴ : 2′ Diphosphate.*—Benzylidine cytidine (1 g.) was phosphorylated with 2·2 mols. of diphenyl chlorophosphonate (1·4 c.c.), and the product (1·9 g.) isolated in exactly the same manner as in the previous experiment. This *compound* was a light-brown amorphous powder which could not be crystallised; it dissolved in alcohol and chloroform but not in water or dilute acid (Found: C, 59·8; H, 4·6; N, 5·4; P, 7·9. $C_{40}H_{35}O_{11}N_3P_2$ requires C, 60·4; H, 4·4; N, 5·3; P, 7·8%).

Hydrolysis and Hydrogenation of 3′ : 5′-Benzylidene Cytidine Tetraphenyl-N⁴ : 2′ Diphosphate. Isolation of a Deaminated Nucleotide.—A mixture of benzylidene cytidine tetraphenyl diphosphate (1·8 g.), methyl alcohol (40 c.c.), and 1·25N-hydrochloric acid (10 c.c.) was refluxed gently for 3 hours. After the hot solution had been shaken with silver oxide (precipitated from 9·8 c.c. of 1·25 N-silver nitrate) and filtered from silver chloride to remove most of the hydrochloric acid, the filtrate and washings were evaporated under reduced pressure to about 10 c.c.; some material which came out of solution was redissolved by adding methyl alcohol (20 c.c.). The solution was shaken at room temperature with hydrogen at atmospheric pressure, Adams's catalyst being added in five portions; 903 c.c. of hydrogen were absorbed (Calc. for hydrogenolysis of 4 phenyl groups, 845 c.c.). The catalyst was filtered off and washed with warm 60% methyl alcohol. After the remainder of the chloride ions had been precipitated quantitatively with silver acetate solution and the acetic acid removed by repeated evaporation under reduced pressure, the solution was diluted with water (200 c.c.) and made alkaline to phenolphthalein with

barium hydroxide solution. The presence of ammonia was recognised by odour and confirmed with Nessler's reagent. Barium phosphate was filtered off, and excess of barium hydroxide was removed from the filtrate and washings by passing carbon dioxide, boiling, and filtering. The filtrate was evaporated under reduced pressure to 25 c.c., and the solid precipitated by addition of absolute industrial alcohol (50 c.c.) was collected by centrifuge, washed with alcohol and ether, and dried. When redissolved in water (25 c.c.) and reprecipitated as before, the deaminated *nucleotide* (0·15 g.) formed a white amorphous powder which failed to crystallise (Found: N, 5·8; P, 6·6; Ba, 32·3. $C_9H_{11}O_8N_2PBa$ requires N, 6·3; P, 7·0; Ba, 30·9%). On hydrolysis with N/10-sulphuric acid, 20% of the phosphorus was liberated as inorganic phosphate in 7 hours.

Hydrogenation of an Equimolecular Mixture of Phenyl Phosphate and Cytidylic Acid.—Disodium phenyl phosphate (5 g.) was dissolved in N-sulphuric acid (50 c.c.), and absolute industrial alcohol (500 c.c.) added. Sodium sulphate was filtered off, and the filtrate and washings evaporated under reduced pressure to 100 c.c.

6·75 C.c. of this solution (equivalent to 48 mg. of phosphorus and 139 c.c. of hydrogen for hydrogenolysis of the phenyl group) and cytidylic acid (0·5 g., equivalent to 26·3 mg. of ammonia from the amino-group) were mixed, and the solution diluted to 30 c.c. with water. When shaken at room temperature in one atmosphere of hydrogen, 0·2 g. of Adams's catalyst being added in two portions, this solution absorbed 135 c.c. of hydrogen in 1½ hours. After removal of the catalyst, analysis of aliquot portions showed the presence of inorganic phosphate (25 mg. of phosphorus) and free ammonia (25·9 mg.). Deamination thus occurred preferentially.

Cytidine-2′ Phosphate.—Preliminary experiments, not described, were carried out to determine the number of phosphoryl groups entering into organic combination, the number removed by acid hydrolysis, and the optimum duration of hydrolysis. A solution of dry benzylidene cytidine (3 g.) in dry pyridine (30 c.c.) was added in 6 portions during ½ hour to a mechanically stirred solution of freshly distilled phosphorus oxychloride (2·8 c.c.) in dry pyridine (10 c.c.), cooled in ice–salt and protected from atmospheric moisture. The reaction mixture was maintained at a low temperature for 3 hours, the temperature gradually rising to 0° and the colour changing through deep yellow to orange-red. The solution was again cooled to −18°, and 50% aqueous pyridine (20 c.c.) was added in 6 portions during ½ hour; a brown gum separated, and the mixture was kept in the freezing mixture for a further 1 hour. After the addition of water (500 c.c.) pyridine was removed under reduced pressure and the solution (180 c.c.) was mixed with N-sulphuric acid (60 c.c.) and refluxed for 2 hours, poured while hot into a boiling suspension of finely powdered silver sulphate (14 g., equivalent to 98% of the chloride ions present) in water (500 c.c.), and, after being shaken vigorously for ½ hour, freed from silver chloride by filtration. Sulphate and phosphate were removed from the combined silver-free filtrate and washings by making alkaline (phenolphthalein) with hot barium hydroxide solution and collecting and washing the precipitate with hot water. The filtrate and washings were evaporated under reduced pressure, and during the evaporation sulphuric acid was added until the solution was just acid to phenolphthalein; barium sulphate was removed and the solution extracted with ether to remove any remaining benzaldehyde. The solution (pH 7—7·5) was concentrated to 40—50 c.c., and filtered 25% lead acetate solution (at pH 7—7·5 with ammonia) added slowly until no more precipitate formed; excess of lead acetate dissolved the lead salt. The lead salt was collected, washed well with water, suspended in water (200 c.c.), and decomposed by passing hydrogen sulphide for 2 hours. After boiling, filtration from lead sulphide, and aeration, the filtrate was evaporated under reduced pressure and mixed with barium hydroxide solution until alkaline to phenolphthalein, excess of barium hydroxide being then removed by passage of carbon dioxide, boiling, and filtration. After evaporation under reduced pressure to 60 c.c., the solution was filtered and mixed with absolute industrial alcohol (120 c.c.). The precipitated barium salt was collected, washed with alcohol, dissolved in water (60 c.c.), reprecipitated, and washed as before; it was crystallised from boiling water (12 parts). The *barium* salt formed small white shining needles (Found in material dried at 100°/0·1 mm.: C, 23·4; H, 2·9; N, 9·1; P, 6·7; Ba, 29·7. $C_9H_{12}O_8N_2PBa$ requires C, 23·6; H, 2·6; N, 9·2; P, 6·8; Ba, 29·9%. Loss from air-dried material: 14·6, 16·5% of dry weight; $4H_2O$ requires 15·7%). The air-dried substance had $[\alpha]_D^{26°}$ + 5·5° (*c*, 1·39 in water).

Cytidine-2′ phosphate was prepared from the barium salt (0·4 g.) by removing the barium quantitatively from the aqueous solution, evaporating under reduced pressure to 10 c.c., adding hot absolute alcohol (20 c.c.) to the boiling solution, and allowing the mixture to cool slowly. The crystals (0·2 g.) were collected and recrystallised by dissolving in boiling water (10 c.c.) and adding hot absolute alcohol (20 c.c.) as before.

Cytidine-2′ Phosphate formed anhydrous white irregular prisms (Found: C, 33·3; H, 4·4; N, 13·0; P, 9·8. $C_9H_{14}O_8N_3P$ requires C, 33·4; H, 4·4; N, 13·0; P, 9·6%). It melted with vigorous decomposition at 240—242° (placed in bath at 230°, diameter of m. p. tube 1 mm., rate of heating 4° per minute), and had $[\alpha]_D^{18°}$ + 21·4° (*c*, 1·05 in water).

For comparison, the following properties of cytidylic acid and of its barium salt are quoted (Levene, *J. Biol. Chem.*, 1919, **39**, 80; 1920, **41**, 484; Thannhauser and Dorfmüller, *Ber.*, 1918, **51**, 467; *Z. physiol. Chem.*, 1919, **104**, 65; Bredereck and Richter, *Ber.*, 1938, **71**, 718). Acid: rectangular plates, m. p. (decomp.) 227°, 230°, 230—233°; $[\alpha]_D^{20°}$ + 48·5°, 47·5° (*c*, 2·0); $[\alpha]_D^{20°}$ + 49·1°. Air-dried salt in aqueous solution: $[\alpha]_D^{20°}$ + 14·0° (*c*, 4·0).

Hydrolysis in 0·1N-Sulphuric Acid at 100°.—(*a*) Cytidylic acid, m. p. 230—233°, was recrystallised thrice from 50% alcohol (Found: C, 33·4; H, 4·4; N, 12·7; P, 9·4. Calc. for $C_9H_{14}O_8N_3P$: C, 33·4; H, 4·4; N, 13·0; P, 9·6%). It (56·2 mg.) was dissolved in water, 0·975 N-sulphuric acid (10·28 c.c.) was added, and the solution diluted to 100 c.c. After removal of 2 control samples the remainder of the solution was transferred to a 250 c.c. "Pyrex" flask and brought rapidly to boiling. The flask was stoppered and immersed in a boiling water-bath up to the level of the liquid inside. At intervals samples were removed, cooled quickly to room temperature, and analysed for inorganic phosphate (Briggs, *J. Biol. Chem.*, 1922, **53**, 13); duplicate determinations had a maximum variation of 4%.

(*b*) The crystalline barium salt of cytidine-2′ phosphate (106·8 mg.) was dissolved in water, and

0·975N-sulphuric acid (12·72 c.c.) was added. Barium sulphate was filtered off and the solution diluted to 120 c.c. and treated exactly as above. Duplicate analyses had a maximum variation of 3%.

	Average dephosphorylation, %.							
Hours at 100°	0	2	4	6	8	20	22	24
Cytidylic acid	0	14	27	36	45	78	83	87
Cytidine-2′ phosphate	0	2	4·5	6·5	8·5	18	19·5	21

Hydrolysis in 0·1N-*Sodium Hydroxide at* 100°.—(*a*) A solution of cytidylic acid (63 mg.) in 0·998N-sodium hydroxide (12·4 c.c.) was diluted to 120 c.c. with water. The solution was treated exactly as described for the acid hydrolysis above; a greenish tinge in the colour developed for the phosphate determinations decreased the accuracy, the maximum variation being 7%.

(*b*) A solution of cytidine-2′ phosphate (70 mg.) in 0·998 N-sodium hydroxide (13·5 c.c.) was diluted to 130 c.c. with water. The solution was treated exactly as described above; here again a greenish tinge decreased the accuracy, the maximum variation being 8%.

	Average dephosphorylation, %.							
Hours at 100°	0	1	2	3	4	5	7	8
Cytidylic acid	0	2	3·5	5·5	7	9	14	16·5
Cytidine-2′ phosphate	0	2	4	6·5	8	11	16	18·5

Electrometric Titrations.—These were carried out according to the procedure described by Gulland, Jordan, and Taylor (*J.*, 1947, 1131).

It is a pleasure to record our gratitude to the Government Grant Committee of the Royal Society, and to Imperial Chemical Industries Ltd., for assistance. Our thanks are also due to Dr. D. O. Jordan for the electrometric titrations, and to Mr. J. E. Still for micro-analyses.

UNIVERSITY COLLEGE, NOTTINGHAM. [*Received, October 9th,* 1947.]

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307. *The Constitution of Yeast Ribonucleic Acid. Part XIII. The Synthesis of Diuridine-2′ : 2′ Phosphate.*

By (the late) J. MASSON GULLAND and H. SMITH.

The first synthesis of a dinucleoside phosphate, diuridine-2′ : 2′ phosphate, is described. It is relatively stable towards alkali and acid, and it is concluded that the presence of phospho-diester linkages is unlikely to be the cause of the facile alkaline fission of yeast ribonucleic acid.

IN previous papers (Gulland and Smith, *J.*, 1947, 338; previous paper) we have shown that the stabilities of nucleoside-2′ and -3′ phosphates towards alkali do not differ significantly, and hence that the facile alkaline fission of yeast ribonucleic acid is not the result of a much greater lability of phospho-ester groups in position 2′ as compared with those in 3′ as has long been postulated, but must be due to some as yet unknown property of the polynucleotide.

The further study of this question by synthetic methods requires the preparation of di-, tri-, and poly-nucleotides containing phosphoryl linkages in such positions, *e.g.*, 2′, 3′, 5′, and N, as might be expected in the nucleic acid molecule, and comparative studies of their hydrolyses with those of yeast ribonucleic acid and other natural polynucleotides. Factors of potential importance also are the relative stabilities of phospho-di- and -tri-esters and the mutual influence of adjacent phosphoryl groups.

The synthesis of *diuridine-2′ : 2′ phosphate* (II) now described indicates that the stabilities of phospho-diesters in this series are unlikely to differ markedly from the related monoesters.

Two mols. of 3′ : 5′-benzylidene uridine (Gulland and Smith, *J.*, 1947, 338) were condensed with one mol. of phenyl dichlorophosphinate in anhydrous pyridine to give a good yield of *di-(3′ : 5′-benzylidene uridine-2′ : 2′) phenyl phosphate* (I).

Attempts to remove the phenyl residue by hydrogenolysis with Adam's platinum catalyst resulted in a simultaneous hydrogenation of the benzylidene radicals which could not then be easily removed by acid hydrolysis (cf. Brigl and Müller, *Ber.*, 1939, **72**, 2121).

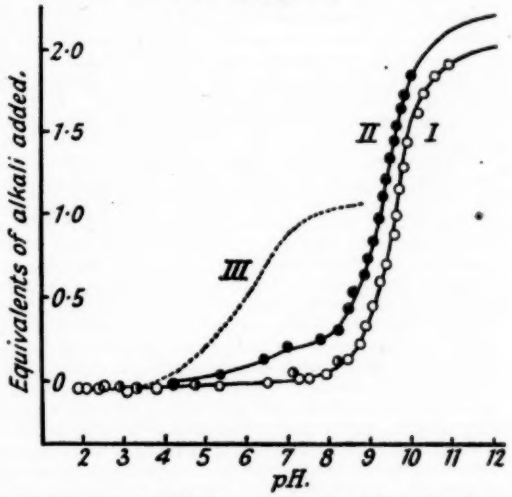
Hydrolysis of the compound (I) with N/2-sodium hydroxide at 100° for ½ hour removed the phenyl residue, and after elimination of the benzylidene radicals by means of hot N/4-sulphuric acid, *barium diuridine-2′ : 2′ phosphate* (as II) was isolated from the mixture in good yield. Purification from small amounts of uridine-2′ phosphate and uridine-2′ phenyl phosphate, inevitably produced in the hydrolysis, was effected by taking advantage of the facts that (i) the lead salt of (II) is not precipitated at pH 7 as is lead uridine-2′ phosphate, and (ii) the barium salt

(as II) is soluble in 66% alcohol but not in 90% alcohol, whereas barium uridine-2′ phosphate is insoluble in the former solvent and barium uridine-2′ phenyl phosphate is soluble in the latter solvent. The barium salt was obtained analytically pure but could not be crystallised and was characterised by its optical rotation, decomposition point, and electrometric titration. Brucine, α-naphthylamine, acridine, *S*-benzylthiouronium, and sodium salts could not be crystallised, and attempts to crystallise the free nucleotide were also unsuccessful.

Electrometric titration of the barium salt (as II) demonstrated the presence of the dissociating groups required for diuridine phosphate, *i.e.*, two enolic hydroxyl groups (pK' 9·7) and no secondary phosphoric acid group.

The hot alkaline hydrolysis of di(benzylidene uridine) phenyl phosphate used in this preparation shows that the ester (II) conforms to the general pattern of phosphodiesters in approximating in stability to the monoesters. Electrometric titrations (see figure) (for details of the method used see Gulland, Jordan, and Taylor, *J.*, 1947, 1131) gave quantitative evidence as to the stability of (II) under conditions which cause complete fission of yeast ribonucleic acid into a mixture of mononucleotides. Titration of the barium salt to pH 1, hydrolysis at 100° for 1½ hours, and back-titration to pH 8 revealed no secondary phosphoryl dissociation, indicating that no significant degree of hydrolysis had occurred. Titration of the barium salt to pH 13, followed by preservation of the solution at room temperature for 24 days and back-titration to pH 4, showed the presence of a small amount (up to 0·2 equivalent) of secondary phosphoryl dissociation, indicating that even in those severe conditions hydrolysis was only very slight.

Electrometric titrations of barium salt of diuridine-2′ : 2′ phosphate.



Full curve I, theoretical curve for diuridine phosphate.

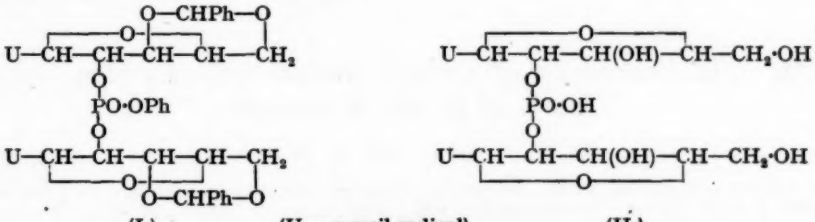
Full curve II, theoretical curve for diuridine phosphate which has undergone hydrolysis with liberation of 0·2 equivalents of secondary phosphoric acid group.

Dotted curve III, theoretical curve for a mixture of uridine phosphate and uridine formed by complete fission of diuridine phosphate.

○ *Titration of diuridine-2′ : 2′ phosphate.*

◑ *Back titration of diuridine-2′ : 2′ phosphate after hydrolysis at* pH 1 *for* 1·5 *hours at* 100°.

● *Back titration of diuridine-2′ : 2′ phosphate after hydrolysis at* pH 13 *for* 24 *days at room temperature.*



(I.) (U = uracil radical) (II.)

It is thus improbable that the presence of phospho-diester linkages as such is the cause of the facile fission of the nucleic acid. Further investigation is envisaged along the lines sketched above.

Experimental.

Di-(3′ : 5′-benzylidene Uridine-2′ : 2′) Phenyl Phosphate.—A solution of freshly distilled phenyl dichlorophosphinate (2·7 c.c.) (Brigl and Müller, *Ber.*, 1939, **72**, 2121) in dry pyridine (50 c.c.) was added during 15 minutes to a mechanically stirred solution of dry benzylidene uridine (10 g.) in dry pyridine (100 c.c.), cooled in ice–salt and protected from moisture. Stirring was continued for 3 hours in the freezing mixture and for 3 hours at room temperature; the reaction mixture attained a deep golden yellow colour. The solution was again cooled in a freezing mixture, 50% aqueous pyridine (50 c.c.) was

added during 15 minutes, stirring was continued for 1 hour, and the reaction mixture poured into ice–water (1·5 l.). Pyridine was removed from the resulting yellow suspension by evaporation under reduced pressure to about 400 c.c., and, after the mixture had been cooled in ice, ice-cold N-sodium hydroxide (100 c.c.) was added; the precipitate dissolved to form a deep orange-red solution which was filtered, N-sulphuric acid was added until the solution was just acid to Congo-red, and the brown precipitate was collected by centrifugation and washed 5 times with ice-cold water. The process of solution and precipitation was then repeated. *Di(benzylidene uridine) phenyl phosphate* (I) (9·3 g.) was collected, washed 5 times with ice-cold water, and dried in a vacuum desiccator over phosphoric oxide; it formed a brown amorphous powder (Found: C, 56·9; H, 4·6; N, 7·0; P, 4·0. $C_{38}H_{33}O_{14}N_4P$ requires C, 56·9; H, 4·4; N, 7·0; P, 3·9%). It formed an intensely yellow solution in alkali which faded on removal of the phenyl radical (see below). It dissolved readily in the usual organic solvents but was practically insoluble in water, dilute acid, light petroleum, and benzene. Attempts to crystallise it have so far failed.

Diuridine-2′ : 2′ Phosphate.—Di(benzylidene uridine) phenyl phosphate (5 g.) was dissolved in N/2-sodium hydroxide (200 c.c.). The intensely orange-yellow solution was refluxed for 30 minutes; the colour faded leaving a faintly brown solution, and analysis for free phenol (Folin and Ciocalteu, *J. Biol. Chem.*, 1927, **73**, 627) showed that over 95% of the originally combined phenol had been liberated. N-Sulphuric acid (200 c.c.) was added, and the solution was refluxed gently for 1 hour, cooled, mixed with N-sodium hydroxide (98 c.c.), and extracted twice with ether (100 c.c.). Sodium sulphate was precipitated, and was removed by evaporation of the solution under reduced pressure to 100 c.c. and addition of absolute industrial alcohol (1 l.). The solution was concentrated and largely freed from alcohol by evaporation with water under reduced pressure. To the solution (50 c.c.), barium acetate (2 g.) was added to precipitate remaining sulphate ions, and then lead acetate (12 g.); the reaction was then adjusted to pH 7 with dilute ammonia, and barium sulphate and a small amount of lead uridine-2′ phosphate were removed and washed with water (20 c.c.). Ammonia was added to the combined filtrate and washings until the pH was 9·5—10·0, and the precipitate was collected by centrifugation and washed 4 times with water (100 c.c.) containing a little dilute ammonia. The lead salt was suspended in water (500 c.c.) and decomposed by passage of hydrogen sulphide for 4 hours. The solution was boiled, filtered from the coagulated lead sulphide, and aerated. After evaporation of the solution under reduced pressure to about 250 c.c., barium hydroxide solution was added until the mixture was alkaline to phenolphthalein, and the excess of barium hydroxide was removed by passing carbon dioxide, boiling, and filtration. Evaporation was then continued to 90 c.c., and absolute industrial alcohol (180 c.c.) was added. 8 Hours later a small precipitate was filtered off (kieselguhr) and washed with 66% alcohol (30 c.c.). Absolute industrial alcohol (800 c.c.) was added to the filtrate, and next day the precipitate was collected, and washed with 90% alcohol. It was redissolved in water (90 c.c.) and the above procedure repeated twice, the final product being washed with alcohol and ether and dried over phosphoric oxide in a vacuum. The *barium* salt (1·6 g.) formed a white amorphous powder (Found in samples of different batches dried at 100°/0·1 mm.: C, 34·8; H, 3·8; N, 9·0; P, 5·0, 5·0, 5·0, 5·0; Ba, 11·2, 11·3, 11·3, 11·0. $C_{36}H_{44}O_{28}N_8P_2Ba$ requires C, 35·0; H, 3·8; N, 9·1; P, 5·0; Ba, 11·1%). When the anhydrous material in a tube of 1 mm. diameter was placed in a bath at 240°, the rise of temperature being 5° per minute, shrinkage occurred at 242° and decomposition with vigorous effervescence at 249—252°. In water different batches of the dried salt had $[\alpha]_D^{13°}$ + 12·2° (*c*, 3·4); $[\alpha]_D^{13°}$ + 12·5° (*c*, 1·6); $[\alpha]_D^{27°}$ + 12·0° (*c*, 2·8).

It is a pleasure to record our gratitude to the Government Grant Committee of the Royal Society, and to Imperial Chemical Industries Ltd., for assistance. Our thanks are also due to Dr. D. O. Jordan for the electrometric titrations and to Mr. J. E. Still for micro-analyses.

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308. *The Conversion of Carbon Dioxide into Acetylene on the Scale of 2—20 Micromoles.*

By W. J. ARROL and R. GLASCOCK.

A method is described for the conversion of carbon dioxide into acetylene in quantities of 50—500 μl. at N.T.P. (2—20 micromoles) with yields generally upwards of 90%. The method is suitable for use with $^{14}CO_2$ of high isotopic concentration.

THE synthesis of biologically important compounds labelled with ^{14}C presents difficulties in technique, partly because the starting substance is carbon dioxide, and partly because it may be necessary to carry out synthesis on the very small scale of millimoles or less, if products of high specific radioactivity are to be obtained from the small quantities of labelled carbon dioxide available.

One compound important as an intermediate in several syntheses is acetylene. Cramer and Kistiakowski (*J. Biol. Chem.*, 1941, **137**, 549) and Vennesland *et al.* (*ibid.*, 1942, **142**, 371) developed methods for converting carbon dioxide labelled with $^{11}CO_2$ into acetylene on the scale of 1—2½ millimoles with yields of about 50—70%. If ^{14}C is to be used in place of ^{11}C,

these methods would entail the disadvantage of handling dry $Ba^{14}CO_3$ and the loss of ^{14}C occasioned by the moderate yield would be serious.

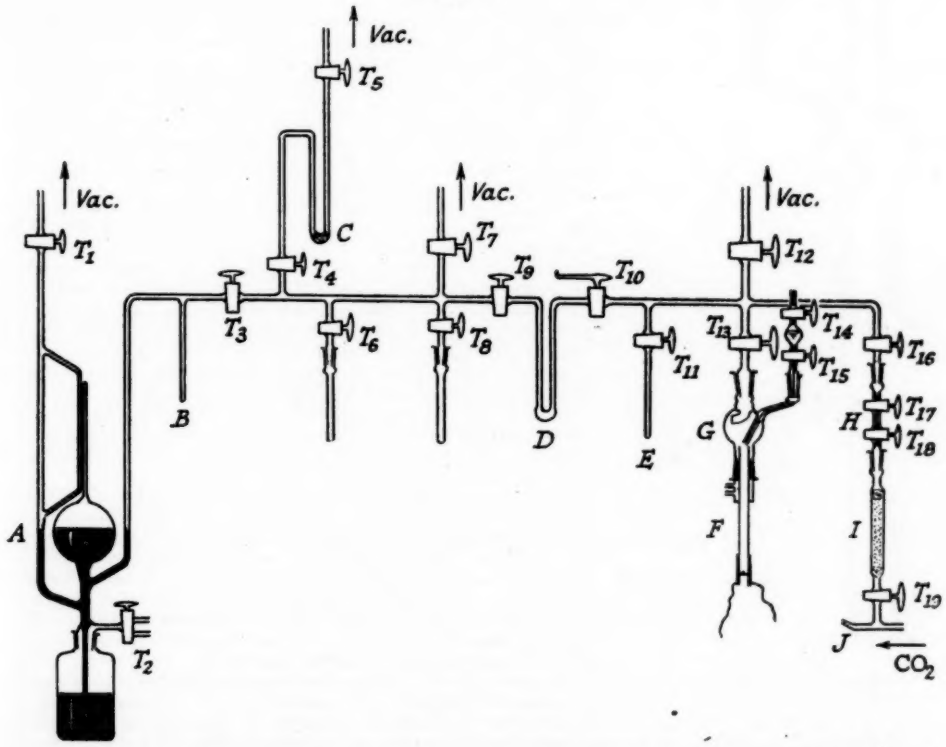
As it is hoped to carry out syntheses in which acetylene is an intermediate, a method has been developed for the conversion of carbon dioxide into acetylene in yields usually of more than 90%, working with quantities of carbon dioxide of 50 to 500 μl. at N.T.P., this being of the order of the volume occupied by a millicurie of isotopically pure $^{14}CO_2$.

It is well known that when calcium is heated suddenly in an atmosphere of carbon dioxide some calcium carbide is formed, and even on a very small scale Chackett (unpublished work) obtained qualitative evidence of the presence of calcium carbide in the residue from a Soddy-type calcium furnace after this had been used to clean up hydrocarbons from inert-gas specimens. In the present experiments, barium heated in a stainless-steel vacuum furnace was found to absorb carbon dioxide to give barium carbide. The contents of the furnace after cooling were dissolved in water, and the acetylene from the barium carbide was separated from the resulting gas mixture.

Experimental.

The high-vacuum apparatus used is illustrated (not to scale) in Fig. 1. The tube *I* was filled with a sample of carbon dioxide from the vaporising solid, the gas sample being dried in *I* with magnesium

Fig. 1.



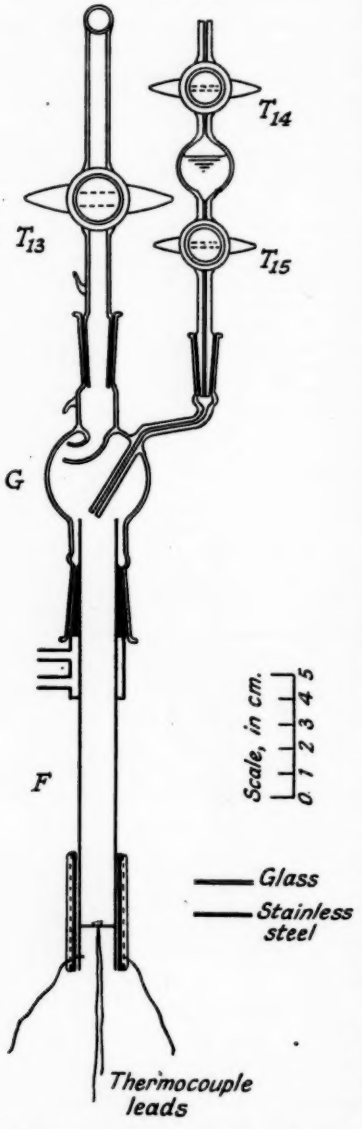
perchlorate. With all stopcocks to vacuum closed, carbon dioxide was admitted to the apparatus in doses through the dosing tube *H*, condensed into the McLeod gauge trap *B*, and, with stopcock T_3 closed, evaporated and its pressure measured so that the volume of carbon dioxide taken could be corrected to N.T.P. In fact, the volume measured was corrected to normal pressure and laboratory temperature since at this stage only relative measurements of gases were required.

A very pure specimen of barium was scraped as clean as possible. About 40 mg. were cut off, weighed, and placed in a stainless-steel vacuum furnace *F* fitted with a splash-head *G*, the whole being evacuated through T_{13} and T_{12} (Fig. 2). With all stopcocks to vacuum closed, the measured specimen of carbon dioxide was condensed into a reservoir *E*, and T_{10} was closed; *E* was warmed to room temperature, and with T_{11} and T_{13} open, the furnace was heated with a bayonet fitting heater wound on a stainless-steel former, the temperature being measured from outside with a platinum–platinum-rhodium thermocouple. It may be seen from Fig. 2 that the design of *F* is such that both the barium and the thermojunction are about at the centre of the heating element. The gas began to disappear at a temperature of about 480°, and in about 5 minutes, by which time the furnace had reached a temperature of 600°, all the gas

except for perhaps 1% or less had disappeared. With the heating element removed, the furnace temperature fell rapidly to room temperature.

The next stage of the process was the decomposition of the contents of the furnace with water. Preliminary analytical experiments on the composition of the mixed gases obtained showed the mixture to consist of a large excess of hydrogen and water vapour together with acetylene, ammonia from the barium nitride on the surface of the barium, and a very small amount of a gas which had physical properties consistent with its being ethylene, although the amount obtained never exceeded 2% of the total C_2 hydrocarbons and was too small for chemica identification. With T_{15} closed, distilled water was introduced into a dosing bulb through T_{14} by means of a narrow metal tube which was then used to pass hydrogen through the water for a few minutes to remove dissolved gases. On withdrawing the tube and closing T_{14}, hydrogen was left above the water surface at 1 atm. pressure. With T_{13} closed, T_{15} was opened cautiously, water passed into F, and the contents of F rapidly dissolved.

FIG. 2.



In preparation for the removal of acetylene from the gas mixture, the charcoal trap C and the rough drying trap D were cooled to $-78°$; T_4, T_5, T_9, and T_{13} were opened while T_3, T_{10}, and all other stopcocks were closed. T_{10} was a control stopcock, having a long handle on the plug and shallow file cuts on opposite sides of the plug leading into the bore so that it could be opened very gradually.

Tap T_{10} was opened cautiously, and the gas mixture from F passed at low pressure through D, where most of the water vapour was frozen out without carrying down any acetylene with it. The mixture, consisting now of hydrogen, water vapour, acetylene, ammonia, and the trace of ethylene, passed into C, where the last four gases were stripped completely from the hydrogen which was pumped away. By this time the solution in F had boiled and become very cold; T_{10} was therefore closed, and F heated gently with the heating element. The boiling-off process and gas transfer were repeated twice.

With T_5, T_7, and T_9 closed and T_3 and T_4 open, the temperature of C was raised to 0°, and B cooled with liquid nitrogen. At 0°, appreciable proportions of acetylene and ethylene were found to be desorbed from charcoal while all the water vapour and ammonia were still adsorbed. The hydrocarbons therefore were condensed into B, the transfer rate falling off not quite exponentially. In about 10 minutes most of the acetylene had been recovered together with the trace of ethylene, and with T_3 closed the gases could be evaporated into the McLeod gauge at room temperature and measured.

The McLeod gauge used had a bulb of 250-ml. capacity and a closed capillary 20 cm. long by 3 mm. internal diameter. An accuracy of about $\pm$ 1—2% was obtainable with amounts of gas varying from 2 to 500 μl. at N.T.P.

Methods of identification and analysis of the gases involved in this work included measurement of vapour pressures at very low temperatures and chemical absorption in standard reagents in the reaction vessels below T_6 and T_8. Carbon dioxide was absorbed in a wet pellet of potassium or sodium hydroxide, ammonia in viscid metaphosphoric acid, and acetylene in a paste of cuprous chloride and potassium hydroxide solution. With all but very small amounts of acetylene, absorption in this reagent gave a red precipitate of copper acetylide.

Results.—Some typical results are given below. Volumes of gases quoted are at N.P. and laboratory temperature ignoring dead space of the McLeod gauge. The volume measurements are relatively accurate and, because the dead space correction and temperature correction are in opposite senses, very nearly at N.T.P.

CO_2 taken, μl.	$C_2H_2(+C_2H_4)$ found, μl.	C_2H_2 in blank.	Yield, %.	CO_2 taken, μl.	$C_2H_2(+C_2H_4)$ found, μl.	C_2H_2 in blank.	Yield, %.
335	178	14	98	62	25·5	1·1	79
273	133	12	89	558	265	3	94
300	140	3	91	566	260	2	91
305	140	3	90	50	21·5	1·8	79

Discussion.—The process described above is a standard procedure which was found to be satisfactory. In earlier work, however, considerable difficulties were encountered. For example, it was found to be important to use stainless-steel as a furnace material. From mild steel, relatively large amounts of acetylene were produced in blank experiments; and a fused

silica envelope fitted with a nickel thimble was found, after it had been used with calcium and barium, to absorb carbon dioxide when hot even though no calcium or barium was present.

The condensation at low temperature of a mixture of acetylene, water vapour, and ammonia all together with mercury (as opposed to adsorption on charcoal) is to be avoided, since acetylene disappears when the trap is warmed, presumably because of the formation of mercury acetylide.

In carrying out the absorption of carbon dioxide in hot barium in the stainless-steel furnace, it was found that when the pressure at which the gas was admitted was less than 10^{-1} mm. of Hg, the yield of acetylene was variable and only about 45%. A similar tendency to low yield (79%) is seen in two experiments where the maximum pressure which could be exerted in our apparatus was lower than usual.

The formation of barium carbide is certainly the result not of a single reaction but probably of several which result partly in the formation of elementary carbon and of barium carbonate from barium oxide and carbon dioxide. Barium carbonate is known to react with hot barium to form carbide and oxide, while elementary carbon intimately mixed with barium might be expected to react appreciably even at 600° by analogy with the direct combination of carbon and calcium investigated by Botolfsen (*Ann. Chim.*, 1922, **18**, 5). If carbon dioxide is admitted to a barium furnace at 600°, at which temperature the barium is distilling quite freely, it will react with the metal vapour, and any elementary carbon or carbonate formed will condense with excess of barium on a cold part of the furnace and will not be reheated with the barium. If, however, the carbon dioxide is present in contact with the barium while this is being heated, vaporisation of the metal will be inhibited and reaction will take place on the surface, the reaction products being further heated in contact with metallic barium. Better yields of carbide would be expected in the second case than in the first.

The scale on which this conversion to acetylene has been carried out is about two orders of magnitude less than has been employed in most syntheses involving $^{14}CO_2$. The scale could fairly easily be increased with probably almost quantitative yields, but could not be decreased very far without the difficulties being encountered of low yield and inconvenience in manipulation and measurement.

We wish to thank Professor W. H. Linnell for his interest and support, many others for helpful conversations, the Research Laboratories of the General Electric Co. Ltd. for a specimen of very pure barium metal, and also to acknowledge generous grants from the Therapeutic Research Corporation, the Royal Society, the Medical Research Council, and the Central Research Fund of the University of London. One of us (R. G.) is a Keddey Fletcher-Warr Student of London University.

COLLEGE OF THE PHARMACEUTICAL SOCIETY,
UNIVERSITY OF LONDON.

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309. *New Syntheses of Heterocyclic Compounds. Part IX. Tetrahydrophenanthridines.*

By B. L. HOLLINGSWORTH and V. PETROW.

Work on the relationship between structure and analeptic activity (Petrow, *J.*, 1946, 200, 884, 888) has been extended by the preparation of some 9-*amino*- and 9-*aminotetrahydrophenanthridines* by direct amination of the corresponding bases.

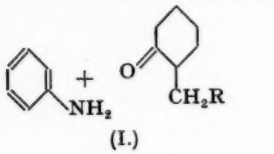
Tetrahydrophenanthridines have been synthesised by (*a*) reaction between 1-diethylaminomethyl*cyclo*hexan-2-one hydrochloride, aniline hydrochloride, and stannic chloride hydrate in boiling *cyclo*hexanone, (*b*) condensation of chloromethyl*cyclo*hexanone, aniline, and aniline hydrochloride in boiling alcohol, (*c*) reduction and ring closure of certain aryliminomethyl-*cyclo*hexanones by heating them with anhydrous formic acid.

A STUDY of the biological properties of the diazaphenanthrenes described in Parts V, VII, and VIII (Petrow, *J.*, 1946, 200, 884, 888) has revealed that certain of these compounds are specific stimulants of the central nervous system, possessing outstanding analeptic properties of the type shown by picrotoxin; but whereas the dose of picrotoxin required to counteract increasing degrees of barbiturate anæsthesia follows a logarithmic pattern, a linear relationship holds with the diazaphenanthrenes (Thorp, forthcoming publication).

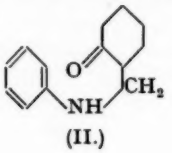
Common features among active compounds of this series appear to be (*a*) a structure analogous to 9-aminophenanthrene, (*b*) a ring nitrogen in position 10, and (*c*) two methyl groups, preferably at positions 1 and 3. Combination of all three factors leads to 9-amino-1 : 3-dimethylphenanthridine, the synthesis of which formed the immediate object of this investigation.

Although this compound was readily formed by the direct amination of 1 : 3-dimethylphenanthridine, the preparation of the latter by the published methods was not entirely satisfactory. This difficulty was overcome by developing a new synthesis of the tetrahydrophenanthridine, from which the required base was readily prepared by selenium dehydrogenation.

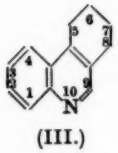
Kenner, Ritchie, and Statham (*J.*, 1937, 1169) have described the preparation of tetrahydrophenanthridine (III) in moderate yield by condensation of hydroxymethyl-*cyclo*hexanone (I; R = OH) with aniline and aniline hydrochloride in boiling alcoholic solution. β-Hydroxy-ketones do not usually react directly with arylamines, and (I; R = OH) gave no evidence of reaction with aniline on prolonged standing at room temperature. At the same time, 1 : 3-ketols are readily dehydrated in the presence of acids to give αβ-unsaturated ketones (cf. diacetone alcohol ⟶ mesityl oxide) which, by virtue of their structure, react with aniline to give β-anilino-ketones. These facts lead to the conclusion that dehydration of hydroxymethyl-*cyclo*hexanone (I; R = OH) to methylene*cyclo*hexanone takes place in the Kenner synthesis (above), followed by addition of aniline to give (II) and ring closure to (III).



(I.)

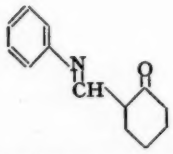


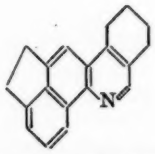
(II.)



(III.)

(IV.)





(V.)

2-Methylene*cyclo*hexanone, recently prepared by Mannich (*Ber.*, 1941, **74**, 554) by high-vacuum distillation of the Mannich base (I; R = NMe_2), is characterised by rapid dimerisation to a *spiro*-compound (*ibid.*, p. 565). Preliminary experiments soon confirmed the highly unstable character of " monomeric methylene*cyclo*hexanone ", and its use in a tetrahydrophenanthridine synthesis was abandoned. An alternative route to compounds of type (II) appeared to lie in the *in situ* preparation of methylene*cyclo*hexanone in the presence of the aromatic base. The practical character of such an approach was first demonstrated by du Feu, McQuillin, and Robinson (*J.*, 1937, 53), who condensed the methiodide of the Mannich base from 2-methyl*cyclo*hexanone with ethyl acetoacetate and sodium methoxide, obtaining the corresponding methyloctalone. Mannich, Koch, and Borkowsky (*Ber.*, 1937, **70**, 355) have carried out a similar condensation using the Mannich base in place of its methiodide.

We now find that diethylaminomethyl*cyclo*hexanone hydrochloride (Mannich and Honig, *Arch. Pharm.*, 1927, **265**, 598) (I; R = NEt_2,HCl) condenses readily with a mixture of aniline hydrochloride, aniline, and stannic chloride hydrate in boiling *cyclo*hexanone to give tetrahydrophenanthridine (III) in 45% yield. There seems little doubt that this reaction proceeds through the intermediate formation of methylene*cyclo*hexanone ⟶ (II) ⟶ (III). Its value lies in the simplicity of the experimental technique. The intermediate (I; R = NEt_2,HCl) does not require isolation, and the complete reaction can be carried out in one vessel by consecutive addition of the appropriate reagents. Other primary aromatic amines (see experimental section) can be equally well employed in place of aniline in this new synthesis, although the reaction fails with 1-naphthylamine, *m*-aminoacetophenone, and 5-aminoacenaphthene.

Since the initial work of Kenner, Ritchie, and Statham (*loc. cit.*), Décombe (*Comp. rend.*, 1941, **213**, 579) has described the conversion of hydroxymethyl*cyclo*hexanone (I; R = OH) into the corresponding chloro-ketone (I; R = Cl). Kenner and Statham (*Ber.*, 1936, **69**, 16) have previously shown that quinolines can be prepared from 2-chloroethyl ketones and primary arylamines. We now find that (I; R = Cl) readily condenses with aniline and aniline hydrochloride in boiling alcoholic solution to give (III), although in only 35% yield. This reaction undoubtedly takes place through the intermediate formation of (II), a fact we have made use of in yet a third route to the tetrahydrophenanthridine system. Compounds such as phenyliminomethyl*cyclo*hexanone (IV) have *trans*-configurations, and thus resist all attempts at ring closure to (III) (see Petrow, *J.*, 1942, 694). Selective reduction of the azomethine linkage of (IV) would clearly give the anilino-ketone (II), readily convertible into (III). We now find that anhydrous formic acid—a reagent enjoying wide use in the reduction of azomethines—will not

only reduce the anil linkage of such compounds as *5-acenaphthenyliminomethyl*cyclo*hexanone*, but will achieve ring closure in the same operation to give the tetrahydrophenanthridine (III). The reaction fails with substituted anilines and 2-aminopyridine, but succeeds with the naphthylamines and 5-aminoacenaphthene (giving V).

9-Aminophenanthridine, *9-aminotetrahydrophenanthridine*, and their *1-methyl*, *3-methyl*, and *1 : 3-dimethyl* derivatives were prepared by reaction of the corresponding bases with sodamide in diethylaniline at 170°. Their biological examination is being reported elsewhere (Thorp, *loc. cit.*).

Experimental.

M. p.s are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford. M. p.s in parentheses are those given by Kenner, Ritchie and Statham (*loc. cit.*).

5 : 6 : 7 : 8-Tetrahydrophenanthridine.—(*a*) *cyclo*Hexanone (50 g.), formalin (9 g.), and diethylamine hydrochloride (11 g.) were heated under reflux until reaction occurred (5 minutes). Heating was then discontinued until the initial exothermic condensation was complete. The crude mixture containing the Mannich base hydrochloride in excess of *cyclo*hexanone was treated with aniline (9·3 g.), aniline hydrochloride (12·8 g.), and stannic chloride hydrate (13 g.), and heated under reflux for a further 16 hours. The mixture was made alkaline with sodium hydroxide solution and the basic fraction isolated with ether. The fraction, b. p. 110—230°/20 mm., on treatment with picric acid (15 g.) in spirit (150 ml.), gave *5 : 6 : 7 : 8-tetrahydrophenanthridine picrate*, yellow needles from spirit (charcoal), m. p. 212—213° (decomp.) (212—214°, decomp.) (Found : N, 13·4. Calc. for $C_{13}H_{13}N,C_6H_3O_7N_3$: N, 13·6%). The base, liberated from the picrate by treatment with excess of aqueous ammonia, formed colourless plates from light petroleum (b. p. 40—60°), m. p. 63° (64°) (Found : C, 85·1 ; H, 7·0 ; N, 7·4. Calc. for $C_{13}H_{13}N$: C, 85·3 ; H, 7·1 ; N, 7·6%), not depressing the melting point of an authentic specimen prepared by the method of Kenner *et al.* (*loc. cit.*). (*b*) Omission of stannic chloride hydrate in (*a*) reduced the yield to 15%. (*c*) Omission of aniline hydrochloride gave yields of less than 5%.

1-Methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine, white plates from alcohol–light petroleum, m. p. 80—81° (80·5°) (Found : C, 85·5 ; H, 7·6 ; N, 7·0. Calc. for $C_{14}H_{15}N$: C, 85·3 ; H, 7·6 ; N, 7·1%), was prepared from *o*-toluidine. The picrate formed yellow needles from spirit (charcoal), m. p. 201—202° (decomp.) (203—204°) (Found : N, 13·1. Calc. for $C_{14}H_{15}N,C_6H_3O_7N_3$: N, 13·1%). Yield 40%.

2 (or 4)-Methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine, a light yellow oil purified by vacuum distillation (b. p. 210°/20 mm.) (Found : C, 84·6 ; H, 8·2 ; N, 6·9. $C_{14}H_{15}N$ requires C, 85·3 ; H, 7·6 ; N, 7·1%), was prepared from *m*-toluidine. The *picrate* formed long yellow needles from spirit (charcoal), m. p. 226° (decomp.) (Found : N, 13·1. $C_{14}H_{15}N,C_6H_3O_7N_3$ requires N, 13·1%). Yield 15%.

3-Methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine, small white plates from light petroleum (b. p. 60—80°), m. p. 72° (73·5°) (Found : C, 85·3 ; H, 7·7 ; N, 7·2. Calc. for $C_{14}H_{15}N$: C, 85·3 ; H, 7·6 ; N, 7·1%), was prepared from *p*-toluidine. The picrate formed very small yellow spangles from spirit (charcoal), m. p. 231° (decomp.) (231—232°, decomp.) (Found : N, 13·1. Calc. for $C_{14}H_{15}N,C_6H_3O_7N_3$: N, 13·1%). Yield 50%.

7-Methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine, white plates from light petroleum (b. p. 40—60°), m. p. 45° (45°) (Found : C, 85·3 ; H, 7·3 ; N, 6·9. Calc. for $C_{14}H_{15}N$: C, 85·3 ; H, 7·6 ; N, 7·1%), was prepared from aniline and *5-methyl-1-(diethylaminomethyl)cyclo*hexan-2-one hydrochloride, obtained from 4-methyl*cyclo*hexanone. The picrate formed gleaming yellow needles from spirit (charcoal), m. p. 196—197° (decomp.) (195°—196°, decomp.) (Found : N, 13·1. Calc. for $C_{14}H_{15}N,C_6H_3O_7N_3$: N, 13·1%). Yield 12%.

1 : 3-Dimethyl-5 : 6 : 7 : 8-tetrahydrophenanthridine, white plates from light petroleum (b. p. 40—60°), m. p. 50—51° (49·5—50·5°) (Found : C, 85·4 ; H, 7·9 ; N, 6·8. Calc. for $C_{15}H_{17}N$: C, 85·4 ; H, 8·1 ; N, 6·6%), was prepared from *m*-xylidine. The picrate formed light yellow needles from spirit (charcoal), m. p. 212—213° (decomp.) (212·5°, decomp.) (Found : N, 12·3. Calc. for $C_{15}H_{17}N,C_6H_3O_7N_3$: N, 12·7%). Yield 20%.

1 : 4-Dimethyl-5 : 6 : 7 : 8-tetrahydrophenanthridine, yellowish white needles from light petroleum (b. p. 40—60°), m. p. 63—64° (63—63·5°) (Found : C, 85·3 ; H, 8·1 ; N, 6·6. Calc. for $C_{15}H_{17}N$: C, 85·4 ; H, 8·1 ; N, 6·6%), was prepared from *p*-xylidine. The picrate formed yellow needle clusters from spirit (charcoal), m. p. 181—182° (decomp.) (180—181°) (Found : N, 12·5. Calc. for $C_{15}H_{17}N,C_6H_3O_7N_3$ N, 12·7%). Yield 20%.

1-Methoxy-5 : 6 : 7 : 8-tetrahydrophenanthridine, yellowish plates from light petroleum (b. p. 80—100°), m. p. 106° (Found : C, 78·5 ; H, 7·2 ; N, 6·6. $C_{14}H_{15}ON$ requires C, 78·7 ; H, 7·0 ; N, 6·6%), was prepared from *o*-anisidine. The *picrate* formed mustard-yellow prisms from spirit (charcoal), m. p. 214° (decomp.) (Found : N, 12·9 ; $C_{14}H_{15}ON,C_6H_3O_7N_3$ requires N, 12·7%). Yield 25%.

3-Methoxy-5 : 6 : 7 : 8-tetrahydrophenanthridine, white needles from light petroleum (b. p. 60—80°), m. p. 109—110·5° (110—111°) (Found : N, 6·4. Calc. for $C_{14}H_{15}ON$: N, 6·6%), was prepared from *p*-anisidine. The picrate formed yellow needles from spirit (charcoal), m. p. 240—241° (241—242°) (Found : N, 12·7. Calc. for $C_{14}H_{15}ON,C_6H_3O_7N_3$: N, 12·7%). Yield 50%.

3-Nitro-5 : 6 : 7 : 8-tetrahydrophenanthridine, white spangles from aqueous acetone, m. p. 172—173° (Found : C, 68·6 ; H, 5·2 ; N, 12·1. $C_{13}H_{12}O_2N_2$ requires C, 68·5 ; H, 5·3 ; N, 12·3%), was prepared from *p*-nitroaniline. The *picrate* formed small yellow needles from acetone–spirit (charcoal), m. p. 203° (decomp.) (Found : N, 15·1. $C_{13}H_{12}O_2N_2,C_6H_3O_7N_3$ requires N, 15·3%). Yield 50%.

3-Chloro-5 : 6 : 7 : 8-tetrahydrophenanthridine, small white needles from light petroleum (b. p. 40—60°), m. p. 90° (90°) (Found : C, 71·7 ; H, 5·7 ; N, 6·5. Calc. for $C_{13}H_{12}NCl$: C, 71·5 ; H, 5·6 ; N, 6·4%), was prepared from *p*-chloroaniline. The picrate formed yellow needles from spirit (charcoal), m. p. 213° (decomp.) (214°, decomp.) (Found : N, 12·5. Calc. for $C_{13}H_{12}NCl,C_6H_3O_7N_3$: N, 12·5%). Yield 30%.

3-*Phenyl*-5 : 6 : 7 : 8-*tetrahydrophenanthridine*, felted white masses from aqueous alcohol, m. p. 122—123° (Found : C, 88·2; H, 6·7; N, 5·3. $C_{19}H_{17}N$ requires C, 88·0; H, 6·6; N, 5·4%), was prepared from *p*-aminodiphenyl. The *picrate* formed yellow needles from spirit (charcoal), m. p. 235° (decomp.) (Found : N, 11·4. $C_{19}H_{17}N,C_6H_3O_7N_3$ requires N, 11·5%). Yield 70%.

5 : 6 : 7 : 8-*Tetrahydro*-1 : 2-*benzphenanthridine*.—1-(1-Naphthyliminomethyl)*cyclo*hexan-2-one (50 g.) (Petrow, *loc. cit.*) in anhydrous formic acid (400 ml.) was heated under reflux for 20 hours. Formic acid (300 ml.) was removed under reduced pressure and the residue poured into excess of aqueous ammonia. The precipitated base was collected, dissolved in spirit, and treated with picric acid (60 g.) in spirit (500 ml.), giving 5 : 6 : 7 : 8-tetrahydro-1 : 2-benzphenanthridine picrate, very small yellow spangles from spirit (charcoal), m. p. 218° (decomp.) (218°, decomp.) (Found : N, 12·5. Calc. for $C_{17}H_{15}N,C_6H_3O_7N_3$: N, 12·1%). The base formed white needles from light petroleum (b. p. 60—80°), m. p. 118—119° (118°) (Found : C, 87·6; H, 6·5; N, 6·2 Calc. for $C_{17}H_{15}N$: C, 87·6; H, 6·4; N, 6·2%). Yield 60%.

5 : 6 : 7 : 8-Tetrahydro-3 : 4-benzphenanthridine, long white needles from light petroleum (b. p. 80—100°), m. p. 100—101° (101°) (Found : C, 87·4; H, 6·5; N, 6·2. Calc. for $C_{17}H_{15}N$: C, 87·6; H, 6·4; N, 6·0), was prepared from 1-(2-naphthyliminomethyl)*cyclo*hexan-2-one (Petrow, *loc. cit.*). The picrate formed small yellow needles from spirit, m. p. 252—253° (decomp.) (245—246°, decomp.) (Found : N, 11·8. Calc. for $C_{17}H_{15}N,C_6H_3O_7N_3$: N, 12·1%). Yield 75%.

1 : 2 : 3 : 4-*Tetrahydro*-11-*aza*-6 : 7-*acechrysene* (V), small cream needles from aqueous alcohol, m. p. 134—135° (Found : C, 88·0; H, 6·7; N, 5·5. $C_{19}H_{17}N$ requires C, 88·0; H, 6·6; N, 5·4%), was prepared from 1-(5-*acenaphthenyliminomethyl*)cyclo*hexan-2-one* [orange-yellow needles from spirit (charcoal), m. p. 137—138° (Found : C, 82·2; H, 7·0; N, 5·1. $C_{19}H_{19}ON$ requires C, 82·3; H, 6·9; N, 5·1%), obtained by the condensation of 5-aminoacenaphthene with formyl*cyclo*hexanone]. The *picrate* formed long yellow needles from spirit (charcoal), m. p. 229° (decomp.) (Found : N, 11·8. $C_{19}H_{17}N,C_6H_3O_7N_3$ requires N, 11·6%). Yield 50%.

9-*Amino*-5 : 6 : 7 : 8-*tetrahydrophenanthridine*.—Dry 5 : 6 : 7 : 8-tetrahydrophenanthridine (5 g.), dry diethylaniline (25 g.), and sodamide (6 g.) were heated in a wax-bath at 160—170°. Vigorous evolution of gas took place, and in 15 minutes the mixture set to a buff solid. The mixture was heated for a further 5 hours at 170°, and the solid, when cold, decomposed with water (25 ml.). After standing overnight, the solids (4 g.) were collected and crystallised from aqueous alcohol. 9-*Amino*-5 : 6 : 7 : 8-*tetrahydrophenanthridine* formed small gleaming white plates, m. p. 165° (Found : C, 78·7; H, 7·0; N, 14·3. $C_{13}H_{14}N_2$ requires C, 78·7; H, 7·1; N, 14·1%).

The *acetate*, white needles from light petroleum (b. p. 80—100°), m. p. 212—213° (Found : C, 69·8; H, 6·9; N, 11·0. $C_{13}H_{14}N_2,C_2H_4O_2$ requires C, 69·8; H, 7·0; N, 10·9%), was obtained by dissolving the base in excess hot acetic acid (33%), and just neutralising the solution with ammonia. Yield nearly theoretical.

5 : 6 : 7 : 8-Tetrahydrophenanthridone was obtained when 9-amino-5 : 6 : 7 : 8-tetrahydrophenanthridine (5 g.), in dilute hydrochloric acid (300 ml.), was treated with an ice-cold solution of sodium nitrite (4 g.) in water (40 ml.) with constant stirring. The mixture was heated on the water-bath for 1 hour, neutralised with ammonia, and the precipitated buff solids (4 g.) recrystallised from aqueous alcohol (charcoal), giving small white needles m. p. 272° (Found : C, 78·4; H, 6·5; N, 7·0. Calc. for $C_{13}H_{14}ON_2$: C, 78·4; H, 6·5; N, 7·1%). Yield 75%. (Sen and Basu, *J. Indian Chem. Soc.*, 1929, **6**, 310, record m. p. 273° for 5 : 6 : 7 : 8-tetrahydrophenanthridone.)

9-*Amino*-1-*methyl*-5 : 6 : 7 : 8-*tetrahydrophenanthridine* formed small gleaming white plates from aqueous alcohol, m. p. 121—122° (Found, C, 79·5; H, 7·8; N, 12·9. $C_{14}H_{16}N_2$ requires C, 79·4; H, 7·6; N, 13·2%). Yield 45%.

9-*Amino*-3-*methyl*-5 : 6 : 7 : 8-*tetrahydrophenanthridine* formed very fine white needles from alcohol–light petroleum (b. p. 80—100°), m. p. 169—170° (Found : C, 79·2; H, 7·3; N, 13·3. $C_{14}H_{16}N_2$ requires C, 79·4; H, 7·6; N, 13·2%). Yield 50%. The *acetate* formed small gleaming white plates from alcohol–light petroleum (b. p. 80—100°) (charcoal), m. p. 204—205° (Found : C, 70·6; H, 7·3; N, 10·6. $C_{14}H_{16}N_2,C_2H_4O_2$ requires C, 70·6; H, 7·4; N, 10·3%). Yield 90%.

9-*Amino*-1 : 3-*dimethyl*-5 : 6 : 7 : 8-*tetrahydrophenanthridine* formed fine white needles from alcohol–light petroleum (b. p. 80—100°) (charcoal), m. p. 136—137° (Found : C, 79·2; H, 7·9; N, 12·3. $C_{15}H_{18}N_2$ requires C, 79·7; H, 8·0; N, 12·4%). Yield 60%.

9-*Amino*-5 : 6 : 7 : 8-*tetrahydro*-3 : 4-*benzphenanthridine* formed white needles from alcohol–light petroleum (b. p. 80—100°), m. p. 241—242° (Found : C, 82·2; H, 6·6; N, 11·3. $C_{17}H_{16}N_2$ requires C, 82·2; H, 6·5; N, 11·3%). Yield 80%.

9-*Amino*-1-*methylphenanthridine*.—1-Methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine (10 g.) was dehydrogenated by heating with powdered selenium (10 g.) at 300° for 5 hours. The cooled product was extracted with boiling absolute alcohol and filtered from selenium. The product crystallised from alcohol–light petroleum in white plates, m. p. 95° (95·5°); picrate, m. p. 233—234° (decomp.) (234°, decomp.). The mixed m. p. with 1-methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine (m. p. 80°) was 61—63°. Yield 85%.

1-Methylphenanthridine (7 g.), diethylaniline (25 ml.), and sodamide (8 g.) were heated at 160° for 4 hours. After a few minutes, the mixture had set to a pasty black solid. The product was decomposed with water (15 ml.), left overnight, and the dark brown solid collected and crystallised from alcohol–light petroleum (b. p. 80—100°) (charcoal). 9-*Amino*-1-*methylphenanthridine* formed white needles, m. p. 116—117° (Found : C, 80·8; H, 5·3; N, 14·0. $C_{14}H_{12}N_2$ requires C, 80·7, H, 5·7; N, 13·8%). Yield 50%. The mixed melting point with 9-amino-1-methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine was 90—92°.

9-*Amino*-3-*methylphenanthridine* separated from alcohol–light petroleum (b. p. 80—100°) (charcoal) in clusters of needles, m. p. 170—171° (Found : C, 80·6; H, 5·5; N, 14·0. $C_{14}H_{12}N_2$ requires C, 80·7; H, 5·7; N, 13·8%). Yield 60%. The mixed melting point with 9-amino-3-methyl-5 : 6 : 7 : 8-tetrahydrophenanthridine (m. p. 169—170°) was 150—153°.

9-*Amino*-1 : 3-*dimethylphenanthridine* formed small white needles from aqueous alcohol (charcoal), m. p. 142—143° (Found : C, 80·8; H, 6·3; N, 12·7. $C_{15}H_{14}N_2$ requires C, 81·1; H, 6·3; N, 12·6%).

Yield 55%. The mixed melting point with 9-amino-1 : 3-dimethyl-5 : 6 : 7 : 8-tetrahydrophenanthridine (136—137°), was 118—120°.

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310. *Solution and Diffusion of Gases in Polystyrene at High Pressures.*

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The solubilities of hydrogen and nitrogen in polystyrene have been found to be proportional to the pressure in the range 80—300 atmospheres, and the solubility constants have been determined at temperatures up to 190°.

The diffusion constants of the same gases in polystyrene, at high pressure, have been measured in the range 20—190°, and found to increase exponentially with temperature.

A few diffusion and solubility constants have been obtained for carbon dioxide and ethylene; and the rate of desorption of hydrogen and nitrogen from polystyrene has been studied.

THE existing data on the solution and diffusion of gases in organic high polymers were summarised by Barrer (" Diffusion in and through Solids ", Cambridge Univ. Press) in 1941. These data are almost entirely confined to the permeability of thin membranes of natural rubber or synthetic elastomers to gases, and were obtained by study of the rates of permeation at pressures of the order of 1 atm.

Since 1941, important contributions to the subject have been made by Barrer (*Trans. Faraday Soc.*, 1942, **38**, 322; 1947, **43**, 3), Reitlinger, (*J. Gen. Chem. U.S.S.R.*, 1944, **14**, 420; translated in *Rubber Chem. Tech.*, 1946, **19**, 385), Doty (*J. Chem. Physics*, 1946, **14**, 244), and van Amerongen (*J. Appl. Physics*, 1946, **17**, 972; *J. Polymer Sci.*, 1947, **2**, 381).

Barrer's summary confirms the view of Graham (1866) and later workers that permeation is a three-stage process of sorption, diffusion, and desorption of the diffusing gas. It also confirms, as Wroblewski (1879) had originally suggested, that the process obeys Fick's law, according to which the permeation rate is proportional to the concentration gradient across the diffusion medium; *i.e.*,

$$P = -D\,\mathrm{d}c/\mathrm{d}x \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where P is the permeability, D the diffusion constant or diffusivity, and $\mathrm{d}c/\mathrm{d}x$ the concentration gradient.

Equation (1) refers to the state of steady flow through a membrane. The equivalent form appropriate to a non-stationary state of flow, as studied in the present work (*i.e.*, accumulation of diffusing matter at a given point in a medium as a function of time), is

$$\mathrm{d}c/\mathrm{d}t = D \cdot \mathrm{d}^2c/\mathrm{d}x^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

It is clear that the applicability of (1) and (2) is conditional on a solubility equilibrium existing at the gas–solid interface, *i.e.*, on diffusion in the solid being the rate-controlling process.

The processes of sorption and diffusion are expressed quantitatively in terms of two constants, namely, the diffusion constant, D (cm.^{2}/sec.$^{-1}$), and the solubility constant, s (c.c. of gas/g. of polymer/atm.). The permeability constant, P, which is derived from D and s, is not used in the present work. Because the density of polystyrene is nearly 1·00, the values of s obtained are comparable with values for other gas–polymer systems given in the literature, but expressed in terms of c.c. of gas/c.c. of polymer.

(a) *The Solubility of Gases in Polystyrene.*

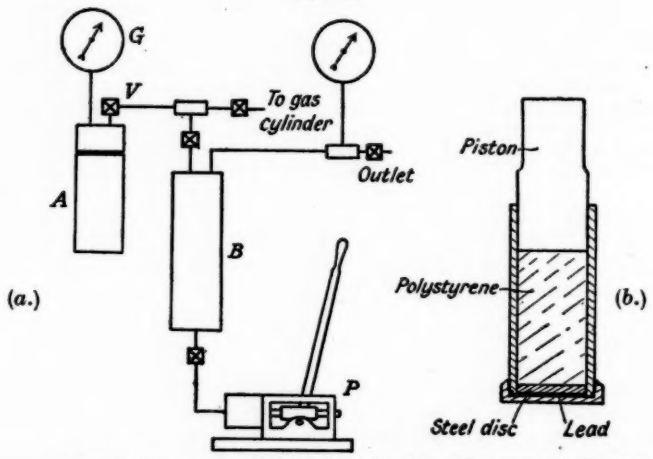
Experimental.—The principle used in the determinations was measurement of the pressure drop, due to gas sorption by the polymer, in a closed system of known volume at constant temperature. The apparatus (Fig. 1) consisted of a stainless-steel pressure vessel, A (capacity about 250 c.c.), the cover of which screwed in to give a metal-to-metal seating. Holes drilled through the cover connected to a gas inlet valve, V, and through copper pressure tubing to a Bourdon pressure gauge, G. The pressure line from A communicated through a T with the gas supply, and with B, a stainless-steel cylinder in which gas could be compressed to the desired pressure by means of the hand-operated mercury pump, P.

The volume of the system was determined by measuring in a gas burette the successive volumes of gas released when a pressure of 2—3 atm. (measured by a large mercury-in-glass manometer) was reduced by stages to 1 atm. Pressure–volume graphs were plotted, and separate runs gave results for the volume agreeing to $\pm$ 0·15 c.c.

The pressure vessel, containing a known weight of polystyrene (40—100 g.) cut into thin discs, was contained in a cylindrical electrically-heated jacket, the space between the two being filled with asbestos wool. Under these conditions, a temperature steady to 1° could be maintained by running the heating jacket at a constant current. For each determination the vessel was kept at a steady temperature for an hour before admission of gas.

The compressed gas was admitted through *V* in about 30 seconds, *V* was closed, and the initial pressure reading was taken one minute later. Blank experiments showed that a constant reading could not be obtained until after this interval, presumably because of the time necessary for attainment of thermal equilibrium. In a typical experiment, the pressure fell slowly for 6—8 hours and then remained constant. Usually the pressure was allowed to reach a constant value overnight, though longer periods were required at temperatures below 130°.

FIG. 1.



(a) *Diagram of apparatus.* (b) *Holder for diffusion experiments (piston in position).*

Discussion.—The gas solubility at the equilibrium pressure and temperature was calculated from the pressure drop and the free volume of the system. In the absence of compressibility data for polystyrene, the volume occupied was calculated on the basis of a density of 1·05 g./c.c. Correction factors at different temperatures for the non-ideal behaviour of the gases were taken from *PV* tables.

The effect of pressure on solubility was examined chiefly at 170° because of the more rapid attainment of equilibrium at this relatively high temperature. It was not possible to obtain higher pressures of ethylene or carbon dioxide than 92·5 and 51 atm., respectively, with the apparatus described.

The data obtained are given in Tables I and II, and shown graphically in Fig. 2. In Table I, solubility (*s*) is recorded in terms of c.c. (measured at N.T.P.) per g., and in Table II, the solubility constant (*s*) is in the units given on p. 1541.

The variation of solubility (corrected for deviations from the ideal-gas law) with pressure is found to obey Henry's law for nitrogen at 170°, hydrogen at 170° and 72°, and ethylene at 170°. Because carbon dioxide could not be used above 50 atm. pressure, it remains uncertain whether the two values found are those for true equilibrium, and whether Henry's law holds for this gas. The corresponding solubility constants (*s*) are :—

Gas.	Temp.	*s*.	Gas.	Temp.	*s*.
Hydrogen.....	170°	0·037	Nitrogen	170°	0·0275
,, ...	72	0·060	Ethylene.....	,,	0·105

Solubility constants, calculated from individual solubility values, were determined for hydrogen and nitrogen over a wide temperature range. No value for nitrogen could be found below 74° as it attained equilibrium much more slowly than hydrogen. The values of *s* for both gases below 120° are not as great as would be expected from the trend of values between 120° and 190°. This may be due to incomplete attainment of equilibrium or to a discontinuity in the solubility–temperature curve, occurring in the softening range of the thermoplastic (95—115°). Experiments on the desorption of gas from previously saturated pieces of polystyrene (described later) indicate minimum values for *s* at 20° to be 0·05 and 0·025 for hydrogen and nitrogen, respectively.

FIG. 2.

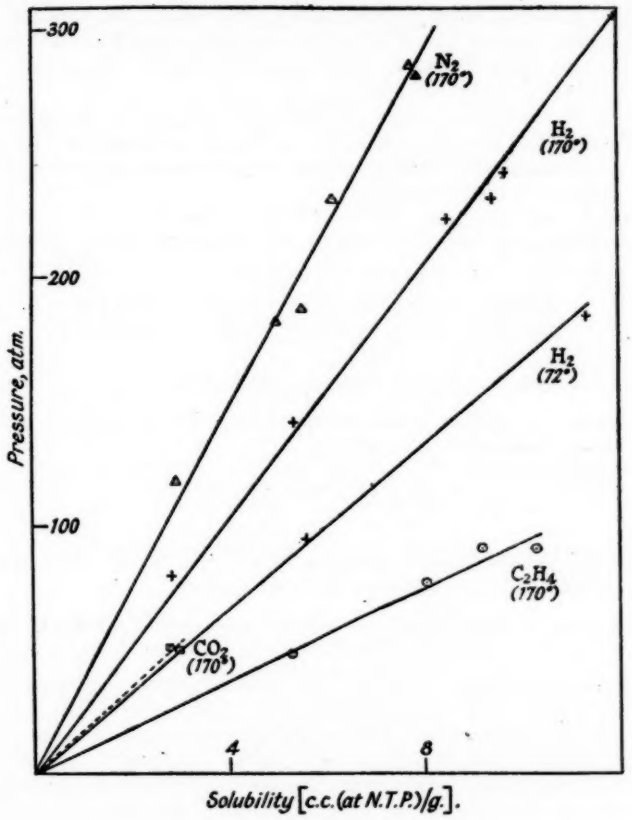


TABLE I.

Solubility data for gases in polystyrene at high pressures and constant temperature.

Press. (atm.).	*s.*	Press. (atm.).	*s.*	Press. (atm.).	*s.*	Press. (atm.).	*s.*
Nitrogen at 170°.		Hydrogen at 170°.		Hydrogen at 72°.		Ethylene at 170°.	
287	7·77	306·5	11·95	186	11·33	92·5	10·30
282·5	7·93	243	9·66	95	5·63	92·5	9·20
232	6·15	233	9·42			77·5	8·05
187	5·57	224·5	8·48	Carbon dioxide at 170°.		49	5·27
182	5·02	142	5·35	51	2·82		
118	2·95	80	2·89	50	3·04		

TABLE II.

Variation of solubility constant with temperature for nitrogen and hydrogen in polystyrene.

Temp.	*s.*	$\log_{10}s$.	$10^3/T$.	Temp.	*s.*	$\log_{10}s$.	$10^3/T$.
	Nitrogen.				Hydrogen.		
191°	0·026	−1·585	2·155	186·5°	0·034	−1·467	2·177
170	0·0275	−1·561	2·257	170	0·037	−1·426	2·257
149	0·031	−1·509	2·370	142	0·042	−1·377	2·410
136	0·033	−1·482	2·445	126	0·047	−1·328	2·506
74	0·035	−1·456	2·882	72	0·060	−1·222	2·899
				49	0·061	−1·215	3·106
				17	0·065	−1·187	3·448

Although, in the works referred to in the introduction, Henry's law has been generally assumed to hold, there have been few independent determinations of sorption equilibria in

organic polymers. The only data recorded for gas–polymer systems at high pressures are those of Tamman and Bochow (*Z. anorg. Chem.*, 1928, **168**, 322), who measured the volumes of hydrogen evolved from different kinds of rubber saturated at 550 and 1150 kg./cm.^{2}. They found Henry's law to be approximately obeyed, with solubility constants of the order of 0·1 (c.c. of gas/c.c. of rubber).

The variation of the solubility constant with the nature of the gas, in polystyrene, is qualitatively similar to that found in rubber at ordinary temperature and pressure. Carbon dioxide and ethylene, having the highest critical temperatures, are most soluble. In rubber, however, the differences are much greater.

For both hydrogen and nitrogen, the solubility constants in polystyrene show a gradual decrease with increasing temperature. Plots of $\log_{10} s$ against $1/T$ in the range 130—190° are approximately straight lines, the equations to which are :

$$\text{For } H_2 \ . \ . \ . \quad \log_{10} s = -2{\cdot}4 + 1900/4{\cdot}6\,T$$
$$\text{For } N_2 \ . \ . \ . \quad \log_{10} s = -2{\cdot}4 + 1700/4{\cdot}6\,T$$

of the form

$$\log_{10} s = \log_{10} s_0 - E_s/2{\cdot}3\,RT \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ (4)$$

in which E_s is the heat of solution in cals./mole; but as the solubility variation is not great, there is considerable experimental uncertainty.

From the relationships :—

$$\Delta G_0 = -RT \log_e s \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ (5)$$
$$\Delta G = E_s - T\Delta S \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ (6)$$

(in which ΔG is the free-energy change), the change in entropy, ΔS, accompanying solution of 1 mole of gas in polystyrene has been calculated as :

for N_2 . . ΔS = (approx.) — 11 entropy units (cals./°c.) per mole at 170°
for H_2 . . ΔS = (approx.) — 10 ,, ,, ,, ,,

These compare with values of from — 8·3 to —10·1 given by Barrer (*Trans. Faraday Soc.*, 1939, **35**, 628) for synthetic elastomers at lower temperatures. It is noteworthy that for water vapour dissolved in polyvinyl chloride–acetate, where specific interaction between polymer and solute undoubtedly occurs, Doty (*loc. cit.*) found $\Delta S = -20{\cdot}8$. This high value was lowered to — 3·6 on addition of 25% of plasticiser. (Doty's units refer to water-vapour pressure of 1 cm. Hg.)

Polystyrene resembles the rubber-like organic polymers in that gas solubilities in it are of much the same order of magnitude as in organic liquids. As with the elastomers, there is no evidence for any chemical or other specific interaction between polystyrene and the gases employed, which are presumably held in solution by van der Waals type forces.

(b) *The Diffusion Constants of Gases in Polystyrene at High Pressures.*

Experimental.—The determination of D by observation of the rate of membrane permeation is clearly not possible at pressure differences much above 1 atm. The method is also restricted to temperatures below the softening point of the plastic.

It was first proposed to calculate D from high-pressure experiments in which the rate of fall of pressure during sorption was observed. This proved impracticable and a method was developed analogous to the determination of the diffusion constant of a coloured substance by observation of the position of the colour boundary. Three variants of the method were used.

(i) *The axial diffusion method.* In this method, used at temperatures above 120°, gas at known pressure and temperature was allowed to penetrate axially through one of the plane faces of a cylinder of polystyrene, for a known time. The polymer cylinder was contained in the cylindrical steel holder shown in section in Fig. 1, and was machined to be a close sliding fit. The bottom of the holder was closed by a brass cap which was obturated by a lead disc.

Before the experiment, the holder containing the polystyrene was heated to *ca.* 150° in an oil-bath, and pressure was applied to the piston in a hydraulic press. This produced a gas-tight join between the polystyrene and the holder. The piston was then removed, and the holder enclosed in the pressure-vessel of the solubility apparatus, which was raised to the required constant temperature. Compressed gas was admitted and allowed to diffuse into the exposed surface for a predetermined time (1 or 2 hours for hydrogen, overnight for nitrogen, carbon dioxide, and ethylene). The pressure was then quickly reduced to 1 atm. (in about 30 secs.), and the holder removed from the pressure vessel.

The polystyrene cylinder, on removal, consisted of a glass-clear lower portion and a cloudy, aerated upper portion into which gas had diffused. In successful experiments, the boundary between the two regions was sharp, consisting of a plane of small bubbles at right angles to the axis of the cylinder. Control experiments showed that, under these conditions, bubbles of gas were formed on pressure-release at pressures of the order of 2—3 atm. In the calculations, the diffusion limit was taken to be the point at which the gas concentration corresponded to 2 atm. pressure. The depth of diffusion, x, is the difference between the original length of the clear cylinder (dimensions approximately 1″ diameter by 2½″ long) and the length of the clear portion of the part-expanded cylinder.

D was calculated as follows: Assuming Fick's law (equation 2), we have

$$C_{xt} = C_s\left(1 - \frac{2}{\sqrt{\pi}}\int_0^{\beta} e^{-y^2}.dy\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

for diffusion normal to the plane surface of a "semi-infinite" solid. Here C_{xt} is the gas concentration x cm. below the surface t secs. after diffusion commences; C_s is the gas concentration at the surface; and $\beta = x/2\sqrt{Dt}$, where D is the diffusion constant in cm.^{2} sec.$^{-1}$.

C_{xt} was taken, as stated above, to correspond to 2 atm. pressure and, as already shown, actual pressures could be used instead of concentrations; x is the depth of diffusion measured as described above.

Equation (7) may be rewritten as

$$C_{xt} = C_s(1 - \text{erf}\, y) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

where erf y is the Gaussian error function; erf y was obtained from (8), the corresponding value of β found from tables and D computed.

(ii) *The modified axial diffusion method.* Below 120° bubbles did not form readily in the polystyrene on release of pressure because of its increased viscosity. It was then necessary to modify the method by heating the holder and polystyrene in an oil-bath to 140—150° (time taken, about 10 mins.), after pressure release, in order to develop the bubble structure. D was calculated as in the first method.

This modification was successful with nitrogen, but few values for D_{H_2} below 120° were obtained by its use. Most of the experiments with hydrogen were spoiled by gas-leakage between the polystyrene cylinder and the steel holder.

(iii) *The radial diffusion method.* This was used to obtain values of D_{H_2} below 80°. At these temperatures, the thermoplastic is rigid and the holder could be dispensed with. A cylinder of polystyrene, machined to the same dimensions as those used in the other experiments, was placed in the pressure vessel, and compressed hydrogen was allowed to diffuse into the flat and curved surfaces. After pressure release, the cylinder was pushed into the steel holder, and the bubble structure developed by heating in an oil-bath.

The holder prevented expansion radially, the increase in volume due to bubble formation being taken up by length-wise expansion. After cooling, sections were cut through the cylinder near the middle, *i.e.*, where the flow is radial, so that the radius of the glass-clear core, to which the gas had not penetrated, could be measured.

D was calculated as follows (Barrer, *op. cit.*, p. 32): For a circular cylinder of radius $r = a$ as diffusion medium, with a constant surface concentration of gas C_s, and with the medium initially free of solute, the solution of the appropriate Fick diffusion equation can be given in terms of Bessel's function of the first kind and zero order $J_0(x)$ and its differential $J_0'(x)$ $[= -J_1(x)]$. The solution is

$$C_{rt} = C_s\left(1 + \frac{2}{a}\sum_1^{\infty}\frac{1}{a_n}.\frac{J_0(a_n r)}{J_0(a_n a)}.e^{-Da_n^2 t}\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

where r is the radius of the clear core to which no gas has penetrated, and a_n is the nth root of the equation

$$J_0(a_n a) = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

TABLE III.

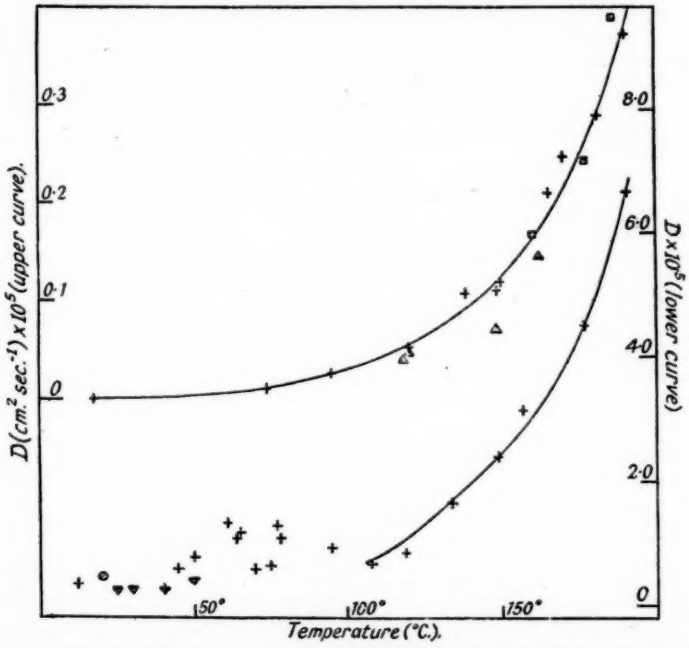
Diffusion constants for nitrogen, carbon dioxide, ethylene, and hydrogen in polystyrene.

Temp.	P (atm.)	$D \times 10^5$.	$\log_{10}D$.	$10^3/T$.	Method.*
		Nitrogen.			
190°	270	0·363	−5·400	2·160	(i)
181	280	0·293	−5·533	2·203	
170	280	0·25	−5·602	2·257	
160	282	0·169	−5·772	2·309	
149	110	0·11	−5·959	2·370	
148	200	0·119	−5·925	2·375	
138	272	0·106	−5·975	2·433	
120	206	0·052	−6·284	2·545	(ii)
95	160	0·027	−6·569	2·717	
74	205	0·01	−7·0	2·882	
18	134	~0·0004	−8·5	3·436	
		Carbon Dioxide.			
186	50	0·39	−5·409	2·179	(i)
177	40	0·245	−5·612	2·222	
165	40	0·211	−5·676	2·283	
		Ethylene.			
210	65	0·644	−5·191	2·070	(i)
162	56	0·147	−5·833	2·299	
148	52	0·071	−6·148	2·375	
120	54	0·048	−6·319	2·545	
118	53	0·042	−6·377	2·558	
		Hydrogen.			
191°	108	6·68	−4·175	2·155	(i)
177	205	4·50	−4·347	2·222	
157	108	3·15	−4·502	2·326	
149	108	2·41	−4·618	2·370	
134	106	1·66	−4·780	2·457	
119	14	0·90	−5·046	2·551	
108	95	0·68	−5·168	2·625	(ii)
95	80	0·96	−5·018	2·717	
75	31	0·65	−5·187	2·874	
40	62	0·29	−5·538	3·195	
78	88	1·10	−4·959	2·849	(iii)
77	94	1·3	−4·886	2·857	
70	104	0·59	−5·229	2·915	
65	43	1·2	−4·921	2·959	
64	108	1·1	−4·959	2·967	
61	98	1·35	−4·870	2·994	
50	102	0·8	−5·097	3·096	
45	96	0·6	−5·222	3·145	
12	96	0·35	−5·456	3·509	

* (i) = Axial diffusion method; (ii) = modified axial diffusion method; (iii) = radial diffusion method.

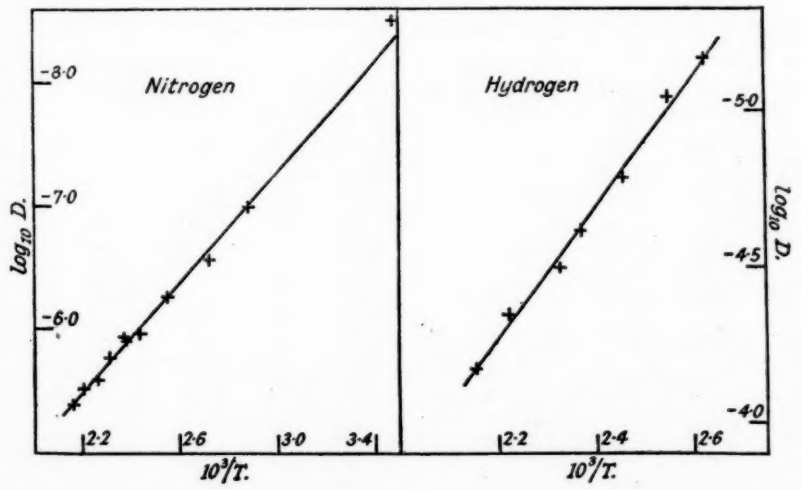
The first three roots of (10) are $\alpha_1 = 2{\cdot}405/a$, $\alpha_2 = 5{\cdot}520/a$, $\alpha_3 = 8{\cdot}654/a$, and these were found to be sufficient. Evaluation of (9) using these roots, gave for each case an equation in three exponential terms which was solved graphically for D. The units used were cm. and secs. with pressures substituted for concentrations in the same way as for the other methods.

FIG. 3.



The upper curve shows the N_2 *diffusion constants* (+), *with the values for* CO_2 (⊡) *and* C_2H_4 (△) *plotted on the same axes. The lower curve shows the* H_2 *diffusion constants* (+). *The points* ⊙ *and* ▽ *represent values taken from the work of Reitlinger, and of Thomas and Gent respectively.*

FIG. 4.



Values found by the radial diffusion method are slightly less accurate than the others because of the greater uncertainty in the radius measurement involved. The results are given in Table III, where D is in the units cm.^{2} sec.$^{-1}$, and shown in Figs. 3 and 4.

Discussion.—The values of D are comparable with those given in the literature for diffusion of gases in rubbers and in organic liquids. The values for hydrogen are much greater, at a given

temperature, than those for nitrogen, whilst the few values obtained for the other two gases approximate to those for nitrogen. The values of D for all the gases increase steeply with temperature.

The diffusion constants of gases in various liquid and solid substances, *e.g.*, organic liquids, glasses, silica, rubber, and synthetic rubbers, have been shown (see Barrer, *op. cit.*) to increase exponentially with absolute temperature, according to the relation

$$D = D_0 e^{-E_D/RT} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

where D_0 is a constant and E_D is the heat of activation of diffusion in cals./mole.

The graphs of $\log_{10}D$ against $1/T$ show that this relation holds for nitrogen in polystyrene and for hydrogen in polystyrene over the temperature range 108—191°, at high gas pressures.

The equations derived from these straight lines are :

For nitrogen : $D = 0{\cdot}21e^{-10,100/RT}$; $\log_{10}D = -0{\cdot}67 - 10,100/2{\cdot}3RT$
For hydrogen : $D = 2{\cdot}04e^{-9,600/RT}$; $\log_{10}D = 0{\cdot}31 - 9,600/2{\cdot}3RT$

As may be seen from the tables, the pressure used in the experiments varied over a fairly wide range, without noticeably affecting the diffusion constants.

The equations above can be compared with those given by Barrer (*op. cit.*) and van Amerongen (1946, *loc. cit.*) for diffusion of nitrogen and hydrogen in rubbers. In the latter, E_D varies between 5100 and 9900 for hydrogen, and between 7200 and 12,400 for nitrogen. The values of D_0 vary between 0·053 and 54·4 for hydrogen, and between 0·22 and 105 for nitrogen.

There are no comparable data for gas diffusion in polystyrene or similar long-chain thermoplastics. The recorded data for D_{H_2} in polystyrene (none for D_{N_2} have been found) appear in Fig. 3. They consist of a value determined by Reitlinger (*loc. cit.*), at 20°, and three derived from data of Thomas and Gent (*Proc. Physical Soc.*, 1945, **57**, 324), which are of uncertain accuracy because the thickness of the films used could not be exactly measured.

The " non-specific activated diffusion " characteristic of gas flow in rubber-like high polymers is now generally assumed to proceed by the following mechanism. Gas molecules dissolve in the surface layer of the polymer and move about inside by jumping into " holes " in their immediate neighbourhoods. These " holes " are constantly forming and disappearing as a result of the random thermal motion of segments of the long-chain molecules. When a concentration gradient exists, the net effect is a drift of gas molecules into regions of low concentration. Capillary flow occurs only as a result of mechanical injury or imperfection.

According to this view E_D is chiefly the energy of " hole " formation. For polystyrene, E_D is greater for nitrogen than for hydrogen. This relation has been found for the rubber-like polymers and is attributed to a molecule of larger diameter requiring a slightly larger " hole " for its passage. Barrer points out that viscous flow of long-chain polymers probably proceeds by a similar mechanism, and that the energies of activation for viscous flow of rubbers are of the same order (10 k.-cals./mole) as E_D.

This view of diffusion is obviously applicable to polystyrene, as the polymer is a non-crystalline disordered tangle (" sub-microscopic felt ") of long-chain molecules.

Ferry (*J. Amer. Chem. Soc.*, 1942, **64**, 1330) has estimated an activation energy of 12 k.-cals. for viscous flow of polystyrene. Also, as would be anticipated from the above view of the diffusion process, Reitlinger found his value of D_{H_2} at 20° to be independent of the average chain length of the polystyrene used.

The Constant D_0.—Several attempts have been made to relate D_0, which varies widely among different media, to more fundamental quantities, such as molecular dimensions, but with little success. Barrer (1942, *loc. cit.*) and van Amerongen (1946, *loc. cit.*) (see also Doty, *loc. cit.*) have pointed out that for rubber and synthetic elastomers an approximately linear relation exists between $\log D_0$ and E_D. The diffusing gases carbon dioxide, nitrogen, and oxygen fall on one line, while hydrogen, which diffuses more quickly, falls on another line. A similar empirical relation may hold for thermoplastics, but insufficient data are available to confirm this.

The diffusion constants of hydrogen in polystyrene below 108° are greater than would be expected from the trend at higher temperatures, and the plot of $\log_{10}D$ against $1/T$ departs from linearity below this temperature. This is not attributable to the lesser accuracy of the results of the radial diffusion method (compare the results of Thomas and Gent, and of Reitlinger).

The values of D_{N_2} obey the exponential relation very well down to 74°. At 18°, the value of D_{N_2} should, according to this, be very small—of the order of 1×10^{-9} cm.^{2} sec.$^{-1}$. An experiment at this temperature, by the modified axial diffusion method, resulted in a gas penetration of ~ 1 mm. in 66½ hours at 134 atm. pressure, giving a value of the expected order for D.

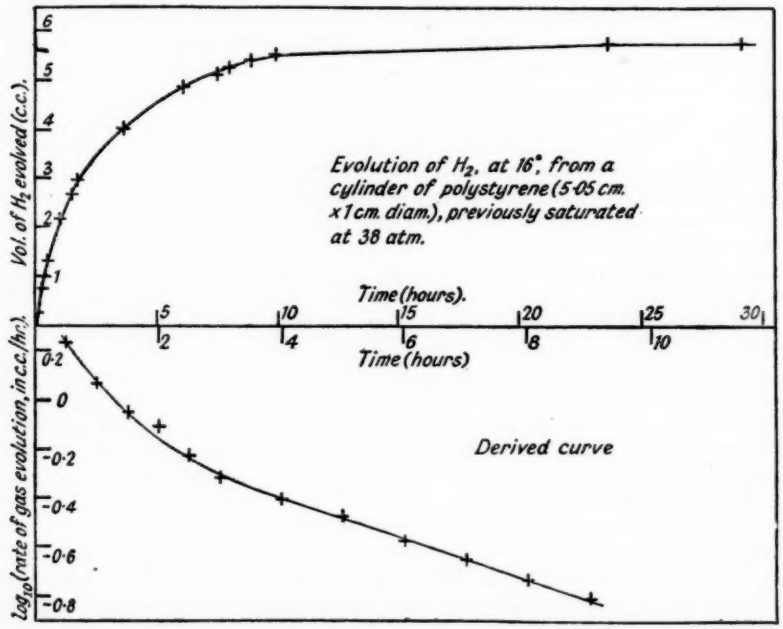
The desorption experiments (see Section c), indicate a value for hydrogen of $D = 1{\cdot}0 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ at room temperature, in fair agreement with the other experimental values. For nitrogen, however, the rate of desorption corresponds to $D = 0{\cdot}1 \times 10^{-5}$, which is much greater than the other values.

A possible explanation for these discrepancies might be that below the softening range of the polymer some form of gas flow, through extremely small cracks or mechanical imperfections, contributes to diffusion. (Visible internal cracks and fracture surfaces are present in pieces of polystyrene, which have been saturated with gas at pressures of the order of 300 atm., and have been released from pressure at temperatures below the softening point.) The small hydrogen molecule might be able to diffuse through these, even when the medium was under high compressive stress, whereas this mode of flow would only become possible for the relatively large nitrogen molecule when the medium was under tensile or "bursting" stress, as in the desorption experiments. The available evidence is too slight to confirm or reject this tentative hypothesis.

(c) *Desorption of Gas from Polystyrene saturated at High Pressure.*

Experimental.—Three experiments with hydrogen and one with nitrogen were carried out. In each case, a machined cylinder of polystyrene was saturated with compressed gas at room temperature. It was necessary, for experimental reasons, to use different pressures for the two gases. After pressure

FIG. 5.


Vol. of H_2 evolved (c.c.).
Evolution of H_2, at 16°, from a cylinder of polystyrene (5·05 cm. × 1 cm. diam.), previously saturated at 38 atm.
Time (hours).
Time (hours)
$\log_{10}$ (rate of gas evolution, in c.c./hr.).
Derived curve

release, the saturated cylinder was placed, with the least possible delay (*ca.* 2—5 mins.), under a mercury-filled gas burette. For each cylinder (see example in Fig. 5), a curve of gas volume evolved (V c.c.) against time (t) was obtained. Tangents were drawn to the volume–time curve at selected points and the rates of evolution at various values of t were calculated from their slopes. Graphs of $\log_{10}$ (rate of evolution) against t were constructed.

Discussion.—Euringer (*Z. Physik*, **1935**, **96**, 37) has shown that, for radial diffusion of gas out of a saturated cylinder, the Fick diffusion equation has a particular solution which may be written

$$\log_e q = \log_e 2cD/r + \log_e (\text{exp.} - 5{\cdot}8\, Dt/r^2 + \text{exp.} - 30{\cdot}5\, Dt/r^2 + \ldots) \quad . \quad (11)$$

where q is the rate of gas evolution from the curved surface (in c.c./cm.^{2} per sec.), c is the initial gas concentration (c.c. at N.T.P./c.c. medium), r is the cylinder radius (cm.), t is the time of desorption (secs.), and D is the diffusion constant (cm.^{2} sec.$^{-1}$).

The assumptions made in deriving (11) are : (*a*) That at the commencement of desorption, the gas is uniformly distributed throughout the medium. (*b*) That the rate-controlling stage of the desorption process is Fick-law diffusion of the gas in the medium. (*c*) That the gas concentration at the surface of the medium is zero.

At high t, depending on the value of D/r^2, the series rapidly converges so that (11) reduces to

$$\log_e q = \log_e 2cD/r - 5{\cdot}8\ Dt/r^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

The derived curves of $\log_{10}$ (rate of evolution) against t are all of the expected shape, showing asymptotic approach to a straight line as t increases. This is characteristic of desorption in which the rate-controlling process is Fick-law diffusion within the medium, and, in theory, D may be calculated from the slope ($= -5{\cdot}8\ D/r^2$) of the straight portion of the derived curve. From the experiment with nitrogen and the experiments with hydrogen dissolved at 57 and 54 atm., the " end effect " (due to desorption from the plane surfaces) makes it possible to say only that the diffusion constants are of the order of

$$\left.\begin{array}{l} 1 \times 10^{-5} \text{ for } H_2 \\ 0{\cdot}1 \times 10^{-5} \text{ for } N_2 \end{array}\right\} \text{ at room temperature.}$$

The experiment with hydrogen dissolved at 38 atm. (Fig. 5) was made using a thinner cylinder, in which the " end effect " was much smaller. From this experiment, D_{H_2} at 16° can be fairly accurately estimated at $1{\cdot}0 \times 10^{-5}$ cm.^{2} sec.$^{-1}$.

In three cases, the polystyrene was heated in an attempt to expel residual gas. The total volume of gas evolved gives a minimum value for s at room temperature of 0·05 for hydrogen and 0·025 for nitrogen.

We wish to thank Messrs. B.X. Plastics Ltd. for supplies of polystyrene, and for financial assistance to one of us (K. E. W.), which enabled this work to be carried out.

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311. *The Anionic Nature of the Desyl and the α-Benzoylbenzhydryl Radical as compared with the Cationoid Reactivity of the Triphenylmethyl Radical. The Hydrolysis of Thio-esters.*

By YOUSSEF ISKANDER.

The thio-ethers (R·SZ) and thiols (R·SH) of the desyl and the α-benzoylbenzhydryl radical (Schönberg and Iskander, *J.*, 1942, 90), which were found to be very easily hydrolysed by alkalis giving RH + Z·S·OH, were found to resist the action of boiling alcoholic hydrochloric acid, and when hydrolysed with concentrated sulphuric acid gave also RH + Z·S·OH; on the other hand, the corresponding thiols and thio-ethers with a triphenylmethyl radical, which are very stable towards alkalis, were found to be easily attacked by concentrated sulphuric acid, giving R·OH + Z·SH. Thus, the anionoid reactivity of the former radicals as compared with the cationoid reactivity of the triphenylmethyl radical were the influencing factors in obtaining two different types of acidic hydrolyses of the corresponding thiobenzoates (cf. *Nature*, 1945, **155**, 141).

THE desyl and the α-benzoylbenzhydryl thiols, thio-ethers, and thio-esters having the general structure COPh·CPhY·SZ (where Y = H or Ph; Z = H, Ph, COPh, $CH_2{\cdot}CO_2H$, $CH_2{\cdot}CH_2{\cdot}CO_2H$, or COPh·CHPh) were found by Schönberg and Iskander (*loc. cit.*) to be hydrolysed by alkalis according to the following schemes:

$$Ph{\cdot}CO{\cdot}CPhY{\cdot}SZ + H_2O \longrightarrow Ph{\cdot}CO{\cdot}CHPhY + Z{\cdot}S{\cdot}OH \quad (I.)$$

or (where Y = Ph and Z = Ph or $CH_2{\cdot}CH_2{\cdot}CO_2H$)

$$Ph{\cdot}CO{\cdot}CPh_2{\cdot}SZ + H_2O \longrightarrow Ph{\cdot}CO_2H + CHPh_2{\cdot}SZ \quad (II.)$$

or a mixture of (I) and (II) where Y = Ph and Z = $CH_2{\cdot}CO_2H$. In all cases of (I) the radicals have preferred to receive the H of water, thus showing anionoid reactivity.

The present work includes the hydrolysis of these compounds by acids, and the comparison of the results of their hydrolysis with acids or alkalis with those obtained from the action of acids or alkalis on similar structures but with a triphenylmethyl radical.

In all the cases, the triphenylmethyl derivatives resisted the action of alkalis but showed varying degrees of susceptibility to the attack of acids; the products of hydrolysis were always triphenylcarbinol, so the triphenylmethyl radical has acquired the OH of water, thus showing cationoid reactivity: $CPh_3{\cdot}SZ + H_2O \longrightarrow CPh_3{\cdot}OH + Z{\cdot}SH$, where Z = H, Ph, or Ph_3C (see also Vorländer and Mittag, *Ber.*, 1913, **46**, 3452; 1919, **52**, 413; Lecher, *ibid.*, 1915, **48**, 524).

On the other hand, all the desyl and α-benzoylbenzhydryl derivatives were not hydrolysed by aqueous-alcoholic hydrogen chloride, but their products of hydrolysis with concentrated sulphuric acid were the corresponding methane derivatives which were obtained through their alkaline hydrolyses.

The colourless solution of didesyl sulphide (either of the two forms) (Schönberg and Iskander, *loc. cit.*) in absolute alcohol becomes blue on addition of a few drops of water, and after 7 hours' boiling on the water-bath a partial hydrolysis into deoxybenzoin and benzil occurs. The same products were obtained in presence of HCl:

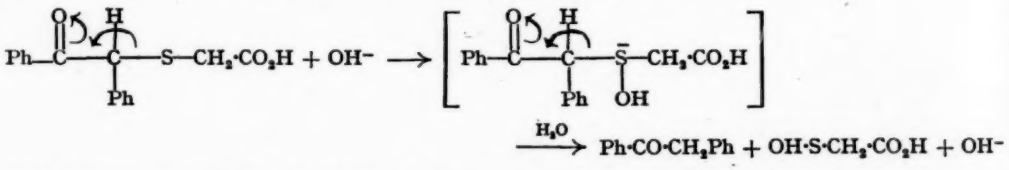
$$(Ph{\cdot}CO{\cdot}CHPh)_2S + H_2O \longrightarrow Ph{\cdot}CO{\cdot}CH_2Ph + Ph{\cdot}CO{\cdot}CHPh{\cdot}S{\cdot}OH$$
$$Ph{\cdot}CO{\cdot}CHPh{\cdot}S{\cdot}OH \longrightarrow Ph{\cdot}CO{\cdot}CSPh \text{ (blue thiobenzil)} + H_2O$$
$$Ph{\cdot}CO{\cdot}CSPh + H_2O \longrightarrow Ph{\cdot}CO{\cdot}COPh + H_2S$$

In the alkaline hydrolysis of didesyl sulphide benzilic acid was isolated as a by-product. The above reaction must have been the side reaction of the alkaline hydrolysis, whereby the benzil was changed into benzilic acid. In the main alkaline hydrolysis reaction the sulphur atom has received two OH groups with a subsequent formation of two molecules of deoxybenzoin, while in the neutral or acid hydrolysis and in the side reaction of the alkaline hydrolysis the sulphur atom has received one OH only. Triphenylmethyl sulphide, on the other hand, is very stable towards alkalis but very easily hydrolysed by concentrated sulphuric acid, the sulphur atom receiving two hydrogens, with subsequent formation of triphenylcarbinol (Vorländer and Mittag, *loc. cit.*).

It seems, therefore, that the susceptibility of the compounds concerned towards acids depends on the proton affinity of the sulphur atom:

$$R{\cdot}SZ + H^+ \longrightarrow \left[R{-}\overset{+}{\underset{H}{S}}{-}Z\right] \xrightarrow{H_2O} R{\cdot}OH + Z{\cdot}SH + H^+$$

while the OH which has its full quota of electrons could not approach the sulphur atom except after that atom has become deficient in electrons by electromeric displacements towards the carbonyl group of the β-keto-thiols and thio-ethers or towards the nitro-group in the *p*-nitrobenzylthio-acids * (Schönberg and Iskander, *loc. cit.*):



And in cases where the alkaline hydrolysis was hindered (scheme II) the sulphur atom must have remained rich with its electrons under the influence of the extra phenyl group and the length of the chain, with a subsequent normal attack of the alkali on the carbonyl group and the elimination of the benzoyl group. The effect of the phenyl group must have also been the cause of the stability of the α-benzoylbenzhydrylthiol as compared with desylthiol (Schönberg and Iskander, *loc. cit.*) and phenacylthiol (Groth, *Centr.*, 1924, I, 1039).

Experimental

Hydrolysis of Triphenylmethyl Thiobenzoate (Vorländer and Mittag, *Ber.*, 1913, **46**, 3452).—(a) *Alkaline hydrolysis.* The thiobenzoate (1 g.), dissolved in alcohol (20 c.c.), was treated with 10% sodium hydroxide solution (10 c.c.), and the mixture boiled on the water-bath for 5 mins. The resulting solution was diluted with water, acidified, and the liberated oil extracted with ether. The ethereal layer, after being washed with sodium hydrogen carbonate solution, contained an oily substance which after solidification had m. p. 107° alone or mixed with an authentic specimen of triphenylmethylthiol. The bicarbonate washings, after acidification, liberated benzoic acid.

(b) *Acid hydrolysis.* The thiobenzoate (1·2 g.), dissolved in boiling alcohol (200 c.c.), was treated with concentrated hydrochloric acid (30 c.c.) and boiled for 15 mins. The colour changed at once to yellow and there was an odour of hydrogen sulphide. Water was then added, and the liberated oil extracted with ether. The ethereal layer, which was yellow, became colourless when washed with sodium hydrogen carbonate solution, and afforded a colourless substance, m. p. 164° alone or mixed with

* Work by Iskander and Riad shows that α-*m*-nitrobenzylthioacetic acid resists the action of alkalis, thus proving that electromeric displacements causing a withdrawal of electrons from the sulphur atom must precede the alkaline hydrolysis.

triphenylcarbinol. The yellow bicarbonate washings, on acidification, liberated an oily acid which was proved to be thiobenzoic acid by oxidation with iodine solution and identity of the product with dibenzoyl disulphide, m. p. and mixed m. p. 133°.

Acid Hydrolysis of α-Benzoylbenzhydryl Thiobenzoate (Schönberg and Iskander, *loc. cit.*).—(*a*) The thiobenzoate (2 g.) in boiling alcohol (200 c.c.) was treated with concentrated hydrochloric acid (30 c.c.), and the mixture boiled on the water-bath for 2 hours. After dilution with water, extraction with ether, and washing with sodium hydrogen carbonate solution, the ether yielded a colourless substance, m. p. 98° alone or mixed with α-benzoylbenzhydrylthiol, and the bicarbonate washings contained benzoic acid.

(*b*) The thiobenzoate (0·1 g.) was dissolved in concentrated sulphuric acid (10 c.c.), and the solution set aside for 5 mins. The resulting blue solution, on dilution with water, precipitated a colourless solid, m. p. 150—154° alone or mixed with di-α-benzoylbenzhydryl disulphide.

Desyl thiobenzoate (Schönberg and Iskander, *loc. cit.*) resisted the attack of boiling alcoholic hydrogen chloride for 6 hours.

Acid Hydrolysis of α-Triphenylmethylthioacetic Acid (Biilmann and Due, *Bull. Soc. chim.*, 1924, **35**, 368).—(*a*) The acid (2 g.) in alcohol (100 c.c.) was treated with concentrated hydrochloric acid (30 c.c.) and water (30 c.c.), and the mixture boiled for 2 hours. After dilution, extraction with ether, and washing with sodium hydrogen carbonate solution to remove unchanged acid, the ether afforded a viscous oily substance which solidified after treatment with few c.c. of alcohol. The solid separated from dilute alcohol in colourless needles of *ethyl α-triphenylmethylthioacetate*, m. p. 94° (Found : C, 76·3; H, 6·2; S, 8·9. $C_{23}H_{22}O_2S$ requires C, 76·2; H, 6·1; S, 8·8%). Hydrolysis of this ester with alcoholic sodium hydroxide solution gave the original acid.

(*b*) The acid (0·1 g.) was dissolved in concentrated sulphuric acid, and the solution set aside for 5 mins. Dilution with water liberated a colourless solid, m. p. 164° alone or mixed with triphenylcarbinol. The acid was recovered unchanged after 2 hours' boiling with 10% sodium hydroxide solution.

Acid Hydrolysis of β-Triphenylmethylthiopropionic Acid (Biilmann and Due, *loc. cit.*).—(*a*) The acid (2·5 g.) was boiled with aqueous alcoholic hydrogen chloride as above for 2 hours. After dilution, extraction with ether, and washing with sodium hydrogen carbonate solution, the ether contained a viscous oil which solidified after treatment with alcohol. The solid separated from alcohol in colourless needles, m. p. 94° alone or mixed with triphenylmethane. The alcoholic filtrate from the oil was shown to contain triphenylmethane and the ethyl ester of the original acid which were separated from each other by hydrolysis with alcoholic alkali.

(*b*) Hydrolysis with concentrated sulphuric acid as with above gave triphenylcarbinol, m. p. 164°. The acid was recovered unchanged after 2 hours' boiling with 10% sodium hydroxide solution.

α-Desylthioacetic acid, β-desylthiopropionic acid, α-(benzoylbenzhydrylthio)acetic acid, and β-(benzoylbenzhydrylthio)propionic acid were treated with aqueous-alcoholic hydrogen chloride as above for 2 hours. Little acid was recovered with sodium hydrogen carbonate, and the ether contained the oily ethyl esters which were hydrolysed with alcoholic sodium hydroxide in the cold to the corresponding starting acids. The benzoylbenzhydrylthio-acids on treatment with concentrated sulphuric acid as above gave a colourless solid, m. p. 136° alone or mixed with benzoyldiphenylmethane.

Acid Hydrolysis of Phenyl Triphenylmethyl Sulphide (Lecher, *Ber.*, 1915, **48**, 524).—(*a*) The substance was recovered unchanged from aqueous-alcoholic hydrogen chloride after long boiling. (*b*) The sulphide, on treatment with concentrated sulphuric acid as above gave triphenylcarbinol, and the filtrate was found to contain thiophenol (oxidised by iodine solution to diphenyl disulphide, m. p. 61°). The substance was recovered completely unchanged after boiling for 4 hours in alcoholic sodium hydroxide solution.

Acid Hydrolysis of Phenyl α-Benzoylbenzhydryl Sulphide (Schönberg and Iskander, *loc. cit.*).—(*a*) The substance was recovered unchanged from aqueous-alcoholic hydrogen chloride after long boiling. (*b*) Treatment of the substance with concentrated sulphuric acid as above gave benzoyldiphenylmethane, m. p. 136° (extracted with ether), and the mother-liquor contained thiophenol. Phenyl desyl sulphide (Schönberg and Iskander, *loc. cit.*) was also found to be very stable towards boiling aqueous-alcoholic hydrogen chloride, but extensively decomposed by concentrated sulphuric acid, giving a deep blue viscous mass with evolution of sulphur dioxide and the odour of thiophenol.

Neutral and Acid Hydrolysis of Didesyl Sulphide (Schönberg and Iskander, *loc. cit.*).—The form of m. p. 169° dissolved in boiling absolute alcohol without any change in colour, but after the addition of few drops of water the solution gradually became blue, but this faded and finally disappeared after 7 hours' boiling. Dilution with water and extraction with ether gave about half of the original substance, m. p. 169° (insoluble in the ether layer), and the ether contained the other form of didesyl sulphide, m. p. 129°, mixed with a yellow viscous substance which, after repeated fractional crystallisation from dilute alcohol, was found to be a mixture of benzil and deoxybenzoin. In presence of hydrochloric acid the same products were isolated. In concentrated sulphuric acid the substance dissolved in the cold with a faint blue colour which became dark blue in the course of ½ hour. On addition of water the blue colour disappeared, and the precipitated oily substance, which partly solidified after some time, was found to contain the original substance, m. p. 169°. The rest of the oil, extracted with ether, was found to be the other form, m. p. 129°. Desylthiol and α-benzoylbenzhydrylthiol dissolve in concentrated sulphuric acid without evolution of hydrogen sulphide, but triphenylmethylthiol liberates the gas in the cold (cf. Vorländer and Mittag, *loc. cit.*). A solution of desylthiol (1 g.) in concentrated sulphuric acid (10 c.c.) was left overnight; water was then added, and the liberated oil was extracted with ether. After evaporation of the ether, alcohol was added and precipitated some free sulphur, m. p. 118—120°. The alcohol-soluble part, after crystallisation from petroleum, proved to be deoxybenzoin.

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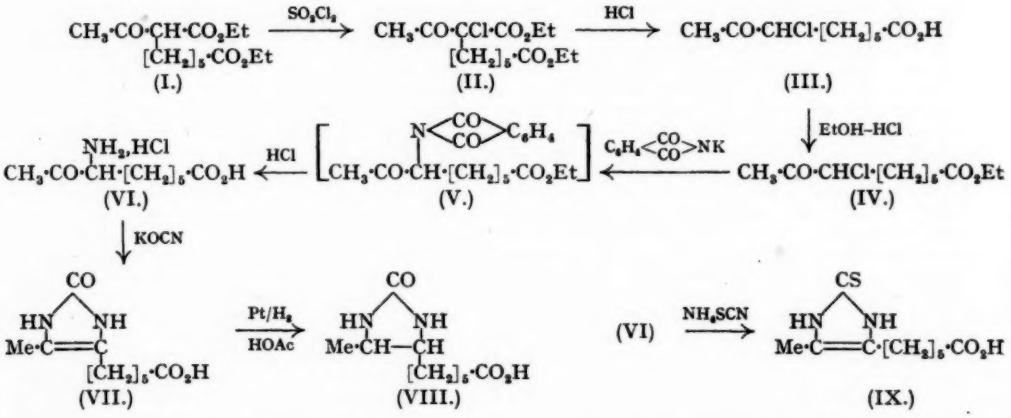
312. *A Synthesis of* DL-*Dethiobiotin and Some Related Compounds.*

By G. SWAIN.

An alternative synthesis of 6-amino-octan-7-one-1-carboxylic acid hydrochloride (VI) affords a modified route to DL-dethiobiotin (VIII). The synthesis of DL-5-(p-*carboxyhexahydrobenzyl*)-4-*methyliminazolid*-2-*one* (XVI), an analogue of dethiobiotin, is described.

SYNTHESES of DL-dethiobiotin (VIII) have been reported by Wood and du Vigneaud (*J. Amer. Chem. Soc.*, 1945, **67**, 210), McKennis and du Vigneaud (*ibid.*, 1946, **68**, 832), Bourquin, Schnider, and Grüssner (*Helv. Chim. Acta*, 1945, **28**, 528), and Duschinsky and Dolan (*J. Amer. Chem. Soc.*, 1945, **67**, 2079). Except the last, in which the pentamethylenecarboxylic acid side chain is introduced into a preformed iminazolone nucleus, all the syntheses proceed through formation of 6-amino-octan-7-one-1-carboxylic acid (VI), or an ester thereof, and differ in the synthetic approach to this compound.

During the course of work directed to the preparation of compounds structurally similar to dethiobiotin in the hope of obtaining biotin antagonists, it has been found that this intermediate amino-ketocarboxylic acid can be prepared through the following reaction sequence :



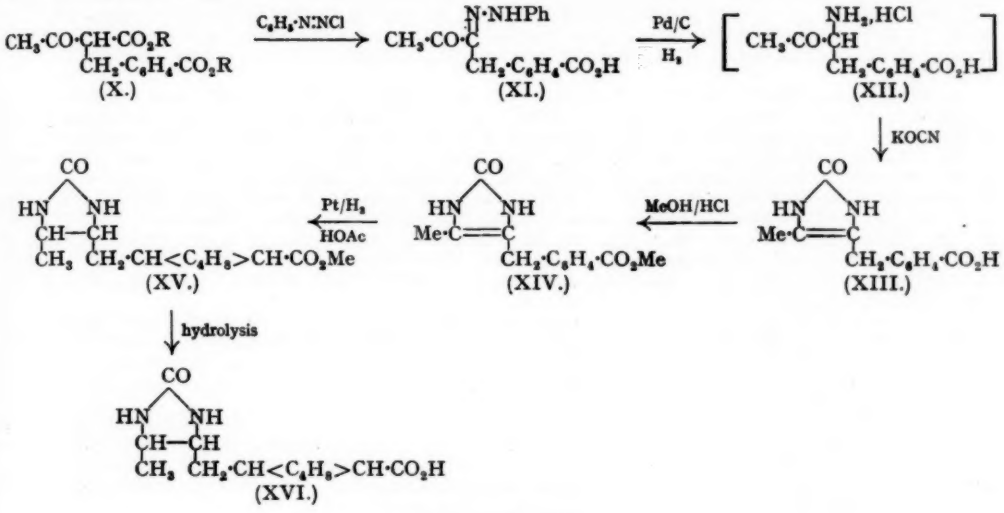
Although it was not possible to isolate the phthalimido-compound (V), yet hydrolysis of the crude reaction product gave (VI), which was readily converted into 4-methyl-5-(ω-carboxy-*n*-pentyl)iminazol-2-one (VII) and the corresponding *thione* (IX) by treatment with potassium cyanate and ammonium thiocyanate, respectively. Hydrogenation of (VII) in acetic acid solution with Adams's platinum catalyst at room temperature and pressure yielded DL-dethiobiotin (VIII) identical with an authentic specimen. Only one isomer was obtained, probably that with the *cis*-configuration at C_4–C_5, since the method of hydrogenation employed is known to favour *cis*-addition to $-C{=}C-$ linkages (cf. Duschinsky and Dolan, *loc. cit.*).

The synthesis of DL-5-(p-*carboxyhexahydrobenzyl*)-4-*methyliminazol*-2-*one* (XVI), a compound closely related to dethiobiotin, but bearing a *cyclo*alkylenecarboxylic acid side chain in place of the *n*-pentylcarboxylic acid side chain present in dethiobiotin, was commenced by a route similar to that described above. Difficulties in the initial stages, however, led to the abandonment of this synthesis in favour of one corresponding to that described by Bourquin, Schnider, and Grüssner (*loc. cit.*) for dethiobiotin, involving the following stages (all substituents in the benzene ring being in the *p*-position) (see p. 1553).

The final hydrogenation with Adams's platinum catalyst in acetic acid solution at room temperature and pressure resulted in absorption of 4 moles of hydrogen and gave rise to a product which was apparently homogeneous. The method of hydrogenation would here again favour the *cis*-configuration in both ring systems.

The compounds (VII), (VIII), (IX), and (XVI) were tested by Dr. J. Madinaveitia for growth-inhibitory activity against *Lactobacillus casei* " in vitro " ; the last two had no inhibitory effect at a concentration of 1/1000. The inhibitory action of DL-dethiobiotin (VIII) (cf. Dittmar, Melville, and du Vigneaud, *Science*, 1944, **99**, 203; Lilly and Leonian, *ibid.*, p. 205; Dittmar and du Vigneaud, *ibid.*, 1944, **100**, 129) was confirmed, and its precursor (VII) shown to possess a

feeble inhibitory action. The inhibition produced by both (VII) and (VIII) was completely reversed by added biotin.



Experimental.

(All melting points uncorrected.)

Ethyl α-Chloro-α-acetylsuberate (II).—Ethyl α-acetylsuberate (27·2 g.; Bourquin, Schnider, and Grüssner, *loc. cit.*) in dry benzene (100 c.c.) was cooled to below 5°, and sulphuryl chloride (15 g., redistilled) added dropwise with stirring during 15 minutes. After a further 15 minutes the temperature was raised slowly to the refluxing point and the mixture maintained under reflux for 15 minutes. Benzene was removed under reduced pressure, and the remaining oil distilled. The fraction, b. p. 134—136°/0·03—0·04 mm. (27·6 g.; 90%), consisted of pure *ethyl α-chloro-α-acetylsuberate* (Found: C, 54·6; H, 7·0; Cl, 12·2. $C_{14}H_{23}O_5Cl$ requires C, 54·8; H, 7·5; Cl, 11·6%).

6-Chloro-octan-7-one-1-carboxylic Acid (III).—The foregoing ester (13·0 g.), glacial acetic acid (20 c.c.), hydrochloric acid (20 c.c., *d* 1·18), and water (5 c.c.) were refluxed for 3 hours, poured into water, and the oil taken up in ether. The ethereal solution was extracted several times with N-sodium carbonate solution. The combined sodium carbonate washings were acidified with hydrochloric acid, and the oil again extracted with ether, washed with water, and dried (Na_2SO_4). Removal of the ether left an oil (9·5 g.) which was distilled. *6-Chloro-octan-7-one-1-carboxylic acid* was obtained as a colourless oil (7·2 g.; 82%), b. p. 128—134°/0·03—0·04 mm. (Found: C, 52·35; H, 7·0; Cl, 16·3. $C_9H_{15}O_3Cl$ requires C, 52·3; H, 7·3; Cl, 17·2%). The *ethyl* ester (IV), obtained by refluxing the acid (85 g., crude) for 6 hours with absolute ethyl alcohol (350 c.c.) containing dry hydrogen chloride (14 g.), was a pale yellow oil (60 g.), b. p. 116—119°/0·15—0·18 mm. (Found: C, 55·5; H, 7·65; Cl, 14·6. $C_{11}H_{19}O_3Cl$ requires C, 56·3; H, 8·1; Cl, 15·1%).

6-Amino-octan-7-one-1-carboxylic Acid Hydrochloride (VI).—The above ethyl ester (5·2 g.), potassium phthalimide (3·6 g.), and xylene (25 c.c.) were stirred under reflux in an oil-bath at 160—165° for 20 hours. The insoluble solid, which remained after dilution with benzene, was filtered off, and the filtrate evaporated under reduced pressure. The residual brown oil was refluxed for 12 hours with concentrated hydrochloric acid (50 c.c.). On cooling, the impure phthalic acid (2·3 g.) which separated was filtered off, and the filtrate evaporated under reduced pressure at 50°. The semi-solid residue (3·8 g.) was dissolved in water (25 c.c.) and filtered through charcoal to remove a little insoluble oil. The clear pale yellow filtrate was again evaporated under reduced pressure at 50°. The crude *hydrochloride* remained as a pale yellow, rather waxy, crystalline residue (3·0 g.; 48%) and was used without further purification for the reaction described below. Recrystallisation from *iso*propyl alcohol was effected with considerable loss to give colourless needles (0·9 g.), m. p. 123—124° (decomp.) (Found: C, 48·65; H, 8·15; N, 6·55. $C_9H_{18}O_3NCl$ requires C, 48·3; H, 8·05; N, 6·3%).

4-Methyl-5-(ω-carboxy-n-pentyl)iminazol-2-one (VII).—Potassium cyanate (2·0 g.) was stirred into a solution of the foregoing hydrochloride (3·0 g.) in water (35 c.c.). A crystalline solid separated rapidly, and after 1 hour hydrochloric acid (10 c.c., *d* 1·18) was added. After standing for 16 hours the suspended iminazolone was collected and washed with water. It was obtained in colourless needles (1·2 g.; 42%), m. p. 163—165° (decomp.) unchanged after recrystallisation from water or ethyl alcohol–ethyl acetate. (Bourquin, Schnider, and Grüssner, *loc. cit.*, give m. p. 167—169°, decomp.; Wood and du Vigneaud, *loc. cit.*, m. p. 168°, decomp.; Duschinsky and Dolan, *loc. cit.*, m. p. 160—170°.) The product slowly decomposes on storage, or on prolonged heating with solvents, becoming brown and resinous. With ferric chloride it gave a maroon and with bromine in carbon tetrachloride a purple colour (Found: C, 56·2; H, 7·1; N, 13·15. Calc. for $C_{10}H_{16}O_3N_2$: C, 56·6; H, 7·55; N, 13·2%).

4-Methyl-5-(ω-carboxy-n-pentyl)iminazole-2-thione (IX).—6-Amino-octan-7-one-1-carboxylic acid hydrochloride (0·8 g., recrystallised), dissolved in water (5 c.c.) mixed with ammonium thiocyanate (0·8 g.), was evaporated to dryness on the steam-bath during ½ hour. Water (7 c.c.) was added twice

to the residue and the mixture evaporated to dryness after each addition. 2N-Hydrochloric acid (6 c.c.) was added, and the mixture set aside for 24 hours. The solid in suspension was collected and washed with water (m. p. 183—185°, 0·55 g., 69%). Recrystallisation from water yielded the *thione* in small colourless prisms, m. p. 184°, undepressed in admixture with a specimen prepared from 6-amino-octan-7-one-1-carboxylic acid hydrochloride obtained by Bourquino, Schnider, and Grüssner's synthesis (*loc cit.*) (Found : C, 53·05; H, 6·65; N, 12·0; S, 13·9. $C_{10}H_{16}O_2N_2S$ requires C, 52·6; H, 7·0; N, 12·3; S, 14·0%).

DL-*Dethiobiotin* (VIII).—4-Methyl-5-(ω-carboxy-*n*-pentyl)iminazol-2-one (400 mg.), dissolved in acetic acid (20 c.c., distilled over chromic acid), was shaken in hydrogen at room temperature and pressure with freshly prepared Adams's platinum catalyst (400 mg.). Reduction was complete in 20 hours. The catalyst was filtered off, and the acetic acid removed under reduced pressure from a water-bath at 45°. The waxy solid residue was crystallised from hot water. DL-Dethiobiotin separated in small rosettes of prisms (250 mg.; 62·5%), m. p. 157—158°, undepressed in admixture with an authentic specimen, m. p. 159—160° (obtained from Messrs. Hofmann La Roche) (Wood and du Vigneaud, *loc. cit.*, give m. p. 157—159°; Bourquin, Schnider, and Grüssner, *loc. cit.*, m. p. 154—155° sintering at 146°, for one isomer obtained from the mother-liquors of the main product, which melted at 134—135°, clear at 145°; Duschinsky and Dolan, *loc. cit.*, give m. p. 162·5—163°) (Found : C, 55·85; H, 8·5; N, 13·0. Calc. for $C_{10}H_{18}O_3N_2$: C, 56·1; H, 8·4; N, 13·1%).

Ethyl p-*Chloromethylbenzoate.*—*p*-Chloromethylbenzoic acid (59 g.; Barkenbus and Holtzclaw, *J. Amer. Chem. Soc.*, 1925, **47**, 2191) was refluxed for 7 hours with alcoholic hydrochloric acid (600 c.c., 2·3%). Excess of alcohol was removed in a vacuum, and the ester isolated in the usual manner. It was obtained as a colourless oil (57 g.; 72·5%), b. p. 146—148°/17 mm. (Einhorn and Papastavros, *Annalen*, 1899, **310**, 205, give b. p. 260—280°).

Ethyl p-*carbethoxybenzylacetoacetate* (X; R = Et). Acetoacetic ester (26 g., redistilled) was added to a solution of sodium (2·3 g.) in absolute ethyl alcohol (40 c.c.). Ethyl *p*-chloromethylbenzoate (20 g.) was added, and the mixture refluxed on the steam-bath for 8 hours. After cooling, it was poured into water, and the oil taken up in ether, washed with water, and dried (Na_2SO_4). Removal of the ether left an oil (38 g.) which was distilled under reduced pressure. The fraction, b. p. 146—148°/0·07 mm. (21·4 g.; 73%), consisted of pure *ethyl* p-*carbethoxybenzylacetoacetate* (Found : C, 65·95; H, 6·95. $C_{16}H_{20}O_5$ requires C, 65·76; H, 6·85%).

1-(p-*Carboxyphenyl*)-n-*butane*-2 : 3-*dione* 2-*Phenylhydrazone* (XI).—The above ester (X; R = Et) (21·9 g.) was added to a solution of potassium hydroxide (9·0 g.; 2·1 equivs.) in water (300 c.c.) cooled to 0—2°. After it had been stirred at this temperature for 24 hours the solution was neutralised (litmus) by addition of N-hydrochloric acid (3·5 c.c.), and sodium acetate solution (26 g. trihydrate in 100 c.c. water) was added, followed by benzenediazonium chloride solution [aniline (7 c.c.) dissolved in 3·75 N-hydrochloric acid (60 c.c.) cooled by addition of ice (80 g.) and diazotised by running in sodium nitrite (5·5 g.) in water (25 c.c.) during 10 minutes; stirred for a further 15 minutes before use]. A buff-coloured solid separated slowly (some frothing). After being stirred in ice for 6 hours, the mixture was allowed to attain room temperature and stirred for 12 hours. The orange-brown solid (18·0 g.) was filtered off, washed with water, and dried at 70°. It crystallised from aqueous ethyl alcohol in yellow prisms (14·1 g.; 63·5%), m. p. 219—220° (decomp., shrinks at 190°). Further crystallisation from ethyl alcohol gave the pure *phenylhydrazone* as yellow prisms, m. p. 222—223° (decomp.) (Found : C, 69·1; H, 5·6; N, 9·55. $C_{17}H_{16}O_3N_2$ requires C, 68·9; H, 5·4; N, 9·5%).

5-(p-*Carboxybenzyl*)-4-*methyliminazol*-2-*one* (XIII).—The above phenylhydrazone (5·9 g., m. p. 219—220°) was suspended in a mixture of methyl alcohol (80 c.c.) and N-hydrochloric acid (42 c.c., 2·05 equivs.) and shaken at room temperature and pressure with palladium–charcoal (1 g., 5%) in an atmosphere of hydrogen. Absorption was complete in 12 hours. The catalyst was filtered off, and the solvent removed under reduced pressure at 30°. The solid residue was dissolved in water (60 c.c.), filtered from traces of insoluble material, and sodium acetate (5·5 g., trihydrate) added to the clear solution. Aniline was removed by extraction with ether. The aqueous solution was freed from ether by warming to 30° under reduced pressure and treated with solid potassium cyanate (2·0 g.). Solid separated slowly and after standing overnight was collected and washed with water (m. p. 330—332°, decomp., shrinks and commences to decompose at 315°); yield 2·5 g. (54%). Crystallisation from aqueous acetic acid (50%) afforded 5-(p-*carboxybenzyl*)-4-*methyliminazol*-2-*one* in very small colourless needles, m. p. 336—338° (decomp; shrinks and commences to decompose at 315°) (Found : N, 11·9. $C_{12}H_{12}O_3N_2$ requires N, 12·1%). The *methyl* ester, prepared by keeping a solution in 5% methanolic hydrochloric acid for 4 days at room temperature, crystallised from methanol in small colourless needles, m. p. 256—258°, with sintering and some decomposition at 240° (Found : C, 63·05; H, 5·7; N, 11·3. $C_{13}H_{14}O_3N_2$ requires C, 63·4; H, 5·7; N, 11·4%).

DL-5-(p-*Carboxyhexahydrobenzyl*)-4-*methyliminazolid*-2-*one* (XVI).—The above methyl ester (1·0 g.) was dissolved in glacial acetic acid (20 c.c.) and shaken with hydrogen at room temperature and pressure in the presence of freshly reduced Adams's platinum catalyst (0·7 g.). Reduction was complete in 3—4 hours (absorption : 335 c.c. at N.T.P. Calc. for $4H_2$, 364 c.c.). The solution was filtered from catalyst, and the acetic acid removed under reduced pressure. The residual oil did not crystallise readily and was hydrolysed by heating at 60—70° for 1 hour with methyl alcohol (10 c.c.) and N-sodium hydroxide solution (16·2 c.c.). Acidification with N-hydrochloric acid (16·2 c.c.) caused separation of the free *acid* as an oil, which crystallised very slowly in colourless prisms (0·55 g.), m. p. 188—190° (sintering at 183—185°). Recrystallisation from water (40 c.c.) yielded nodules of small colourless prisms, m. p. 188—190° (sinter 185°, slightly opalescent melt clearing at about 210°) (Found : C, 59·6; H, 8·15; N, 12·05. $C_{12}H_{20}O_3N_2$ requires C, 60·0; H, 8·3; N, 11·7%).

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313. *Structure of the Dextran Synthesised from Sucrose by a New Strain of* Betacoccus arabinosaceous.

By M. Stacey and G. Swift.

A water-soluble gum-like dextran has been synthesised from sucrose by a new strain of *Betacoccus arabinosaceous* (*Leuconostoc mesenteroides*). The hydrolysis products of the methylated dextran were separated chromatographically and shown to consist of 2 : 3 : 4 : 6-tetramethyl glucose (1 part), 2 : 3 : 4-trimethyl glucose (3 parts), and 2 : 3-dimethyl glucose (1 part). The structure of the repeating unit is thus identical with that described by previous investigators for the dextran from another strain of *L. mesenteroides*. The molecule in these dextrans shows an unusually high degree of branching.

Work carried out in Sweden (Grönwall and Ingelman, *Acta Physiol. Scand.*, 1944, **7**, 97; 1945, **9**; Bohmansson, Rosenquist, Thorsen, and Wilander, *Acta Chir. Scand.*, 1946, **94**, 148) and in this country (A. R. Lockwood and his colleagues, unpublished) has revealed that products from the degradation of dextran may find an important use as substitutes for plasma in blood transfusion and in shock treatment. In a study of numerous dextran types we have isolated an organism, a strain of *Betacoccus arabinosaceous* (*Leuconostoc mesenteroides*) (see Experimental section) which has the power of synthesising a dextran at an unusual rate and in relatively large quantities.

The dextran has some physical properties which distinguish it from other dextrans which we have examined over a number of years in these laboratories. These dextrans, notably those produced by various strains of *L. mesenteroides* and *L. dextranicum*, are all retrograded to a water-insoluble form on dehydration or on being kept for long periods. The dextran described herein is unusual and of interest inasmuch as it forms colloidal solutions of high viscosity and it cannot be converted into a water-insoluble form. In its physical properties it closely resembles some of the plant gums. We have deemed it of interest to examine its structure by the usual methylation technique with application of chromatographic separation in order to relate it to other dextrans.

The problem of the constitution of various dextrans has engaged the attention of numerous workers during the past decade. So far it had been established that all these polyglucoses contain a high proportion of repeating units having α-D-glucopyranose units mainly linked through the 1 and 6 positions. In some dextrans, cross linkages of undetermined location also occur.

An investigation of the structure of the dextran synthesised by a strain of *L. mesenteroides* from sucrose was described by Fowler, Buckland, Brauns, and Hibbert (*Canadian J. Res.*, 1937, **15**, 486) who prepared a methylated dextran and hydrolysed it by treatment with methanolic hydrogen chloride. From the fractional distillation of the resulting glucosides they claimed that the dimethyl, trimethyl, and tetramethyl methylglucosides were present in the approximate ratio of 1 : 3 : 1, and these were identified as 2 : 3-di-, 2 : 3 : 4-tri-, and 2 : 3 : 4 : 6-tetra-methyl methylglucoside respectively. A branched chain structure for the dextran based on these results was proposed. These results were subsequently criticised by Brauns (*ibid.*, 1938, **16**, 73) on the following grounds: (*a*) the dextran was incompletely methylated; (*b*) the ratio of tetra- to tri- to di-methyl methylgucosides of 1 : 3 : 1 was not conclusive because of the inefficient fractional distillation employed; and (*c*) a large percentage (18·4%) of material was lost during fractionation. A reinvestigation of this dextran was made by Levi, Hawkins, and Hibbert (*J. Amer. Chem. Soc.*, 1942, **64**, 1959) in order to establish more definitely its structure. In this attempt to reaffirm the claims in the earlier paper these authors pointed out that in complex polysaccharides such as mannan, glycogen, and araban, which contain intricately branched chains, every branching position must yield a dimethyl methylglycoside in the case of hexosans and a monomethyl methylglycoside in the case of pentosans. Consequently, in methylation studies of such polysaccharides a final methoxyl value of 1—2% lower than the theoretical can render the results worthless with respect to the extent of branching and so preclude an accurate structural assignment. These authors treated dextran with methyl sulphate and alkali, and after three such treatments obtained a partly methylated dextran (OMe, 40—41%) which was further methylated by a modified Muskat technique (*ibid.*,1934, **56**, 693) which consisted of suspending the dextran in anhydrous anisole and treating the resulting suspension with liquid ammonia and sodium. The methylation was then carried out by the addition of methyl iodide to this mixture. After six such treatments the dextran had the theoretical methoxyl content of 45·6% as calculated for $C_6H_7O_2(OMe)_3$. The methylated dextran was hydrolysed with

methanolic hydrogen chloride in sealed glass bombs, and the resulting glucoside mixture was fractionated. By this procedure the ratio of 2 : 3 : 4 : 6-tetra- to 2 : 3 : 4-tri- to 2 : 3-di-methyl methylglucoside was again established as being exactly 1 : 3 : 1, thus verifying their earlier results.

On the above evidence, the authors suggested for dextran two tentative branched chain formulæ (cf. Figs. I and II), differing only in the position of attachment of the side-chain. These, however, do not exhaust all the possibilities, and it is conceivable that the side chain may consist of one, two, or three units with a corresponding lengthening of the primary chains. Three of the linkages between the building units are of the 1 : 6 type while the remaining two are either 1 : 4 or 1 : 6. The only evidence available as to whether the linkages are α- or β- is the change in rotation towards the negative side on hydrolysis of the methylated dextran, *e.g.*, $[\alpha]_D + 200° \longrightarrow + 70°$. The importance of these investigations lies in the fact that they indicated that the dextran synthesised by *L. mesenteroides* from sucrose is a highly-branched structure differing from the linear type of structure of the dextran produced from *L. dextranicum* proposed by Peat, Stacey, and Schlüchterer (*J.*, 1939, 581).

Some claims for the confirmation of these results have been obtained by the study of the *L. mesenteroides* dextran molecule with the electron microscope by Ingleman and Seigbahn (*Nature*, 1943, **152**, 154), who claimed that their electron-micrographs and ultra-centrifuge measurements indicated that the molecule possessed an extremely high molecular weight.

Peat, Schlüchterer, and Stacey (*loc. cit.*) and Stacey and Youd (*Biochem. J.*, 1938, **32**, 1943) investigated the dextran synthesised by *L. dextranicum* from sucrose. These authors obtained a methylated dextran, and from its methanolysis products succeeded in isolating a small percentage (0·23%) of an " end group " (identified by conversion into 2 : 3 : 4 : 6-tetramethyl glucopyranose anilide) which indicated a chain length of not more than 550 glucose units, while osmotic-pressure measurements on the methyl ether indicated a chain length of not less than 200 glucose units. The remaining distillation product consisted of approximately 10% of dimethyl methylhexosides. Efforts were made to identify this fraction; proof was obtained that it was not homogeneous and there was some evidence obtained later that it consisted mainly of 2 : 3-dimethyl methylglucoside and an unidentified dimethyl methylglucoside (E. Schlüchterer, 1940, Ph.D. Thesis, University of Birmingham). This investigation was of particular interest in that it indicated that the structure of the dextran synthesised by *L. dextranicum* was essentially of the straight-chain type, though some as yet undetermined branching does occur.

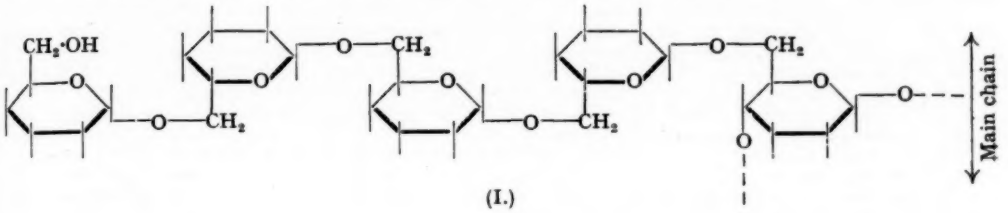
The contemporaneous investigations of Hibbert and his co-workers (*Canadian J. Res.*, 1938, **16**, 151) provided verification of these findings in the *L. dextranicum* dextran. Another interesting dextran is that produced from sucrose by *Betabacterium vermiformé* Ward–Mayer, the production and properties of which were carefully investigated by Mayer (Mayer, " Das Tibi Consortium " Delft, 1938). It was shown (Daker and Stacey, *J.*, 1939, **132**, 585) that this particular dextran consisted of α-glucopyranose units united by 1 : 6-glucosidic linkages in chains which consisted of about 25 glucose units, and the authors suggested that these chains are united to form larger molecular aggregates in possibly the same way as the basal chain units of starch (*i.e.*, amylopectin) are combined.

The new dextran described herein was produced from a sucrose substrate, and it dissolved in water to give clear viscous solutions. It remained gum-like and water-soluble even after being intensively dehydrated over long periods. It was purified by repeated precipitation from aqueous solution with alcohol, and was finally isolated without treatment with acid or alkali as a water-soluble white powder. It was methylated in two 20 g. portions, using methyl sulphate and 30% sodium hydroxide solution, by essentially the method of Peat, Schlüchterer, and Stacey (*loc. cit.*). After two methylations the partly-methylated dextran was purified by precipitation of a chloroform solution with ligroin and was isolated in the form of a pale yellow powder. This product was then thoroughly dried in a vacuum for several days and was then re-methylated by forming the sodio-derivative in liquid ammonia, and treating this with methyl iodide. After 6 additions of sodium with the corresponding quantities of methyl iodide, the product was a white powder.

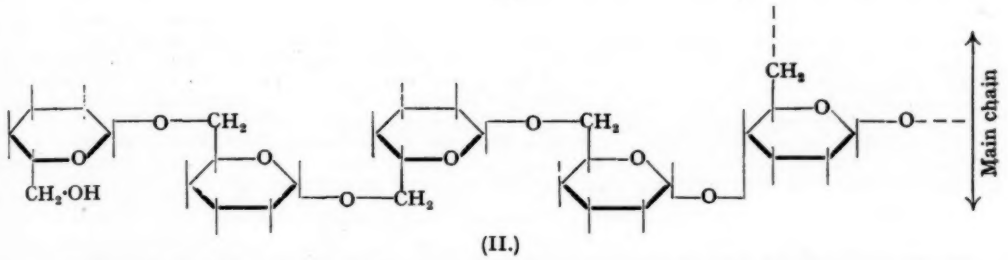
The fully-methylated dextran was hydrolysed by being heated on a boiling water-bath for 7 hours with glacial glacial acetic acid and 5% aqueous hydrochloric acid (3 : 2 by volume). The acids were neutralised and the solution freed from chloride ions by the addition of lead acetate, the lead then being removed with hydrogen sulphide.

The sugars were separated by essentially the method of Bell (*J.*, 1944, 473; cf. Gilbert, Smith, and Stacey, *J.*, 1946, 622) by use of a partition between organic solvents and water in a column of silica gel.

There were isolated and identified 2 : 3-di-, 2 : 3 : 4-tri-, and 2 : 3 : 4 : 6-tetra-methyl glucose in the proportions of 1 : 3 : 1 (see Experimental section), and thus in this respect the constitution of the repeating unit of the methylated dextran was identical with that described by Hibbert and his co-workers (*locc. cit.*) for the dextran produced by the action of their strain of *L. mesenteroides* on sucrose. The type of structure could be represented by (I) or (II) which are essentially the branched chain formulations of Hibbert and his co-workers (1942, *loc. cit.*).



(I.)



(II.)

It appeared, therefore, that the new organism was *L. mesenteroides*, but it was evidently a different strain, because the dextran it produced had physical properties different from those of the dextran from known strains of *L. mesenteroides*.

It is possible that a repeating unit of the type shown in (I) or (II) may be identical with that of the dextrans produced by various strains of *L. mesenteroides*, although it is likely that the branching occurs at different places. Indeed, such small differences as are shown between (I) and (II) and other variations of the type of structure may account for the physical differences between the polysaccharides formed by the various strains. It is likely also that the number of repeating units in each macromolecule is different. It is an interesting finding that even different strains of an organism give dextrans having different physical properties, and it appears that one may eventually be able to identify an organism and its various strains by determining the finer structure of its extracellular polysaccharides.

Experimental.

Characterisation of the Micro-organism (with Mr. A. R. Lockwood).—The organism was originally isolated from an aqueous solution of cane sugar which had become "ropy". The crude ropy sugar contained yeasts, together with a very small coccus and a larger coccus. The larger coccus was obtained in pure culture, gave good dextran production, and was designated *Betacoccus arabinosaceous* Birmingham.

Morphologically, the organism more closely resembles *Betacoccus arabinosaceous* Orla–Jensen in so far as cultural pleomophism is much less marked than that exhibited by *L. mesenteroides* strains. Elongation of cocci into rod forms in old cultures is much less frequent than with *L. mesenteroides*, and streptococcal chains are encountered continuously even in very vigorously agitated cultures.

Growth on sucrose nutrient agar is characteristically raised and "mucoid" at sucrose concentrations in the region of 10%, but thin-spreading films only are produced on media having lower sucrose concentrations, *i.e.*, 1—3%, under the same cultural conditions. *L. mesenteroides* and *L. dextranicum* produce good raised slimy colonies on these media containing low sucrose contents, and continued sub-culture of this new organism on such media fails to produce the pronounced raised slime. This "osmophilic" character of the strain is further encountered in liquid media containing low sucrose concentrations where growth is slow and sparse with poor dextran production.

Attempts to classify the organism by secondary physiological characters as described by Hucker and Pederson have not been wholly satisfactory, and these investigations are proceeding. Pentoses appear to be weakly fermented, a character in which the organism resembles *L. mesenteroides*, although the good vigorous growth obtained in sucrose media has not been obtained with any other carbohydrate source.

The growth-factor requirements of the organism appear to be identical with those of *L. mesenteroides* and *L. dextranicum*, and, under the most favourable conditions of culture so far developed for these organism as a class, the rate of production of acetic and lactic acids, carbon dioxide, and traces of ethanol exhibited by this new strain is indistinguishable from that of *L. mesenteroides* and other related strains.

Production of Dextran.—The culture medium contained the following: microcosmic salt (4·9 g.),

potassium hydrogen phosphate (1·0 g.), potassium chloride (0·5 g.), *p*-aminobenzoic acid (0·5 g.), hydrated magnesium sulphate (0·5 g.), hydrated ferrous sulphate (0·01 g.), peptone (0·01 g.), sucrose (100 g.), and distilled water (1000 c.c.). The liquid was poured in 200 c.c. quantities into penicillin culture jars and sterilised by being steamed for ½ hour on each of 3 successive days. When cold it was inoculated with a viscous suspension (2 c.c. in each jar) of the *Betacoccus* growing in the same medium. The flasks were incubated at 25°. After 24 hours the medium became opalescent and there was a gradual increase in viscosity which reached a maximum in 5—7 days, at the end of which time the medium lost its opalescence.

By gradual addition of ethanol (1 vol.), the dextran was precipitated as a rubber-like mass which gradually lost its elasticity on being dehydrated. It was fractionated by repeated precipitation by ethanol and acetone from water at different pH values until it was obtained essentially ash-free. Traces of extraneous protein were then removed by chloroform–butanol extraction. The main bulk of dextran (35 g.) was obtained as a reasonably homogeneous white granular powder having $[\alpha]_D^{18°}$ + 180° (*c*, 0·57 in water) (Found: N, 0·02; water, 8·2; ash, 1·9%). For larger scale production the dextran could readily be produced in 6 l. amounts in 10 l. jars.

Despite many attempts at denaturation by dehydration using solvents, vacuum drying, etc., the dextran always remained water-soluble, redissolving to give solutions the high viscosity of which was almost unaffected by addition of electrolytes. Addition of Fehling's solution to the dextran solution rapidly precipitated the dextran as a gelatinous mass.

Hydrolysis of the Dextran.—The dextran (5·0 g.) was hydrolysed by N/10-sulphuric acid at 98°, and the following polarimetric changes were observed: $[\alpha]_D^{20°}$ + 180° (0 hours); + 156° (1¼ hours); + 147° (2½ hours); + 142° (3½ hours); + 80° (10 hours); + 52° (20 hours, equilibrium value). The sulphuric acid was neutralised with barium carbonate and the filtered solution evaporated in a vacuum to a syrup which crystallised on being kept and was identified as glucose (5·0 g.). Examination of the mother liquor on a paper chromatogram showed that only glucose was present in the hydrolysate.

A comparison was made of some properties of a number of dextrans produced under the same conditions by different organisms which had been kept for a number of years. The dextrans received the same treatment for their purification and the differences are noted in the Table.

Organism.	$[\alpha]_D^{20°}$.	Found, %. Ash.	N.	H_2O.	Behaviour on dehydration.
Mixed culture from beet sugar "slime"	+147° (in dil. NaOH)	8·3	3·5	8·2	Rapidly becomes completely insoluble in water
L. dextranicum (originally obtained from Prof. A. J. Kluyver)	+180° (in dil. NaOH)	6·2	1·8	8·0	Gradually becomes completely insoluble in water
L. mesenteroides (originally obtained from Prof. A. J. Kluyver)	+180° (in dil. NaOH)	3·1	1·2	7·5	Gradually becomes completely insoluble in water
L. citrovorum (originally obtained from Lister Institute)	+179° (in dil. NaOH)	1·5	0·9	6·2	Swells in water but does not dissolve
Betacoccus vermiforme Ward–Mayer (originally obtained from Prof. A. J. Kluyver)	+178° (degraded material in water)	1·5	0·5	5·8	Rapidly becomes completely insoluble in water
Betacoccus arabinosaceous Birmingham	+180° (in water)	1·9	0·98	8·2	Remains completely soluble giving viscous solutions

It will be seen that the only unusual property of the new dextran is its solubility in water, but this difference is quite striking for it relates it more closely to polysaccharides of the gum or mucilage type.

Methylation of Dextran.—The dextran (20 g.) was dissolved in 30% sodium hydroxide (100 c.c.) and treated at room temperature with 30% sodium hydroxide (250 c.c.) and methyl sulphate (180 c.c.) over a period of 2 hours during which 1/10th of the reagents were added drop by drop every 10 minutes. Acetone in portions of 100 c.c. was added at intervals. The reaction was carried out in an atmosphere of nitrogen in a 5-l. bolthead fitted with a mercury seal. After the addition of the reagents the reaction was allowed to proceed for 1 hour at room temperature. Finally, the temperature was slowly raised to 100° in order to destroy sodium methyl sulphate. The solution was then neutralised (hot) with 5N-sulphuric acid; a white flocculent precipitate of partly methylated dextran then came out of solution. This was filtered off and found to be insoluble in all common organic solvents. However, it swelled in dioxan, and this property was used to facilitate further methylation. A further sample (10 g.) was methylated once by this method, and the two batches of partly-methylated dextran were re-methylated by a similar procedure until a chloroform-soluble product was obtained on neutralisation. This property of being soluble in chloroform enabled it to be purified, for it could be precipitated from the solution by ligroin. The precipitate had OMe, 25·2%. The mother liquors from the three methylations were dialysed until free from sodium sulphate and were then evaporated to dryness. Total weight of product, 28·3 g.

Methylation by Liquid Ammonia–Methyl Iodide.—The apparatus and methods were similar to those frequently used for starch methylation, except that, in order to avoid losses due to the sparing solubility of the partly methylated dextran in liquid ammonia, the ammonia was allowed to evaporate instead of the usual syphoning method being used. As the modified method did not suffice to remove any sodium iodide, the product was purified after 4 additions of sodium. Partly methylated dextran (4 g.; OMe, 25%) so obtained was dried in a vacuum for several hours and was then suspended in liquid ammonia (200 c.c.) in a bath at — 80° (solid carbon dioxide–alcohol). Sodium was added and the mixture was then stirred for 6 hours in order to dissolve the metal; methyl iodide was then added dropwise, and the reaction allowed to proceed for ½ hour after which more sodium was added. At the end of this time the ammonia was allowed to evaporate overnight at room temperature, the last traces being removed by placing the tube in a boiling water-bath. The solid was then boiled with water to remove sodium

iodide, filtered, washed several more times with boiling water, and dried (3·8 g.; OMe, 39·4%). The product was suspended in 200 c.c. of liquid ammonia and re-methylated using sodium (2 g.) and methyl iodide (6·5 c.c.), added in 2 portions.

The product was purified as before, but in addition a further purification was carried out by precipitation of a chloroform solution with ligroin. Yield, 3·5 g.; $[\alpha]_D^{20°}$ + 212° (*c*, 1·1 in chloroform) (Found, on three different samples: OMe, 45·5, 45·9, 45·5%). Fractionation by means of solvents did not separate the product into fractions having different methoxyl contents, optical rotation, or viscosity and the product was considered to be essentially homogeneous.

Hydrolysis of the Methylated Dextran.—The methylated dextran (5·0 g.) was dissolved in glacial acetic acid (60 c.c.), and 5% hydrochloric acid (40 c.c.) was added. The solution was then heated on a boiling water-bath for 7 hours. After cooling, a slight excess of lead acetate was added and the precipitate of lead chloride filtered off and washed with a little ice-cold water. Hydrogen sulphide was then passed through the filtrate and washings, and the filtered colourless solution, after being evaporated to small volume with frequent additions of water and freed from chloride ions with silver carbonate, was evaporated to dryness under reduced pressure. Yield, 4·5 g.

Chromatographic Separation of the Methylated Sugars (Gilbert, Smith, and Stacey, *J.*, 1946, 622).—The following precautions were observed: (*a*) the apparatus was freed from grease and all taps were lubricated with graphite; (*b*) organic solvents were distilled from an all-glass apparatus, and the chloroform was thoroughly washed with water before distillation and stored in a large separating funnel under a layer of water.

Silica. This was prepared according to the method of Gordon, Martin, and Synge (*loc. cit.*), but the addition of an indicator to the silica was omitted.

Silica–water column. The dry special silica was ground in a mortar, and ½ its weight of water was stirred into the powder with further grinding to ensure efficient mixing. A glass tube of suitable dimensions (see later) was loosely plugged at one end by cotton-wool resting on a removable, perforated, porcelain dish. The moist silica was made into a paste with chloroform and poured into the tube. The gel rapidly packed into a column on top of the plug. The supernatant chloroform was allowed to drain through the gel, and the column was then freed from traces of grease by running through successively two column-lengths of chloroform. The apparatus was then ready for use.

Separation and identification of sugars. (I) The sugar mixture (4·49 g.) was dissolved in water (40 c.c.), the solution filtered into a graduated separating funnel, and the final concentration brought to approximately 5% by water-washings. The solution was shaken 9 times with its own volume of chloroform, and the latter evaporated, without dehydration, at ordinary pressure. This extract contained all the tetramethyl glucose and, in addition, about 10% of the trimethyl glucose.

(II) The sugars extracted in (I) were dissolved in chloroform and quantitatively transferred by pipette to a column prepared from 25 g. of silica in a tube of 40 mm. diameter. One chloroform-length was then passed through the column, and the eluate taken down to dryness. Weight of sugars isolated, 1·075 g. (fraction *A*).

(III) The aqueous phase from (I) was evaporated to dryness. Weight of remaining sugars, 3·235 g.

(IV) The sugars isolated in (III) were dissolved in acetone and transferred quantitatively by pipette to the column. One column-length of chloroform–butanol (9 : 1) was passed through the column, and the eluate evaporated, without dehydration, at ordinary pressures. A syrup (1·595 g.) remained (fraction *B*). The remaining sugars were eluted from the column by means of acetone and the eluate taken to dryness. A further syrup (1·610 g.) (fraction *C*) remained; this was more viscous than fraction *B*.

Identification of fraction A. This fraction, which was a mixture of crystals and syrup, was extracted with boiling ligroin. The crystals dissolved leaving the syrup. On evaporation of the ligroin, a crystalline product (0·841 g.) was obtained. After being recrystallised twice from ligroin it had m. p. 88° alone or in admixture with 2 : 3 : 4 : 6-tetramethyl glucose (Found: OMe, 52·8. Calc.; OMe, 52·5%).

From the crystals there was prepared an anilide which after 3 recrystallisations from ether–ligroin had m. p. 125°, undepressed in admixture with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl glucose anilide. The syrup which remained after the extraction with ligroin was later combined with fraction *B* to give fraction *D*.

Conversion of the small amount of fraction *D* into the anilides showed that it consisted mainly of 2 : 3 : 4-trimethyl glucose (anilide, m. p. 140°), while by the same method it was shown that fraction *C* consisted mainly of 2 : 3-dimethyl glucose (anilide, m. p. 119°).

From both fractions most of the 2 : 3 : 4-trimethyl derivative was separated in the form of its β-methylglucoside, m. p. 91° (Found: OMe, 53·0%). The residue was converted into the anilides which were quantitatively separated by fractional crystallisation from ether–ligroin and on a silica gel column into 2 : 3 : 4-tri- and 2 : 3-di-methyl glucose. No other constituents could be detected.

The three methylated glucose derivatives were separated from the hydrolysate from further samples of the methylated dextran (3·2 g.), and it was found that there were present 2 : 3 : 4 : 6-tetra-(0·814 g.), 2 : 3 : 4-tri- (2·495 g.), and 2 : 3-di-methyl glucose (0·883 g.). Thus the molecular ratio of these components is 1 : 3 : 1 respectively.

The authors thank Professor Sir Norman Haworth, F.R.S., for his interest in this work, and one of them (G. S.) thanks the East Anglia Chemical Company for a grant which enabled him to take part in the investigation.

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314. *The Constitution of a Levan produced from Sucrose by* Pseudomonas mors-prunorum (*Wormald*).

By Violet E. Gilbert and M. Stacey.

The polysaccharide produced from sucrose by *Pseudomonas mors-prunorum* (Wormald) is shown to be a typical levan, the repeating unit of which is a terminated chain composed of about twelve fructofuranose units mutually joined through the 2 and 6 positions. Experiments on the chromatography of methylated fructoses are described.

Pseudomonas mors-prunorum is the casual agent of bacterial canker in plum trees (Wormald, *Ann. Appl. Biol.*, 1930, **17**, 725; *Trans. Brit. Mycol. Soc.*, 1932, **17**, 157). Some biochemical characteristics of the organism were studied by Erikson (*Ann. Appl. Biol.*, 1945, **32**, 44, 112, 117), who succeeded in cultivating it on a simple synthetic medium containing sucrose as the sole source of carbon.

Among the more interesting metabolic products produced during the growth were a yellow-green fluorescent pigment and a polysaccharide. The effect of injections of the polysaccharide, both alone and together with the *Pseudomonas mors-prunorum* bacterial cells, on the resistance of certain plum trees to bacterial canker was also studied by Erikson (*loc. cit.*, p. 117). Interesting results were obtained, and the preliminary findings of this mode of approach to problems of bacterial infections of fruit trees appears to be worthy of further development. Miss Erikson kindly placed at our disposal a sample of the polysaccharide, and the present paper describes the investigation of its structure. The polysaccharide was obtained in an essentially homogeneous water-soluble form, $[\alpha]_D$ − 47°, giving opalescent solutions of low viscosity.

On hydrolysis with dilute acid the polysaccharide was shown to be constituted of fructose residues only. The polyfructose was methylated, and on methanolysis gave rise to *ca.* 9% of 1 : 3 : 4 : 6-tetramethyl fructofuranoside " end residue ", the remainder consisting of 1 : 3 : 4-trimethyl methylfructofuranoside. Thus the *Pseudomonas mors-prunorum* polysaccharide contains a repeating unit which is composed of a terminated chain of 10—12 fructofuranose units mutually joined through the 2 and 6 positions. It belongs to the same class as other plant and bacterial levans, notably those from certain plant pathogens (Challinor, Haworth, and Hirst, *J.*, 1934, 1560; Lyne, Peat, and Stacey, *J.*, 1940, 237) which have been investigated previously in these laboratories.

During the course of this work attempts were made to separate various mixtures of tetramethyl and trimethyl fructofuranosides and fructopyranosides by chromatographic methods in order to develop a micro-method of assaying the chain length of fructosans. It has been possible to achieve separation of trimethyl from tetramethyl fructosides in the qualitative sense, but so far a convenient chromatographic technique for separating them quantitatively has eluded us.

Experimental.

Production of the Polysaccharide (D. Erikson).—The basal medium contained the following salts: dipotassium hydrogen phosphate (1 g./l.), magnesium sulphate (hydrated) (0·5 g./l.), and potassium chloride (0·5 g./l.). After being adjusted to pH 7 the solution was autoclaved, and when cold there was added a sterile aqueous solution of sucrose to make a final concentration of 10 g./l.

After inoculation with a culture of *Pseudomonas mors-prunorum* the medium was incubated at 25° for periods up to 20 days. The polysaccharide formed was precipitated from the metabolism solution by addition of several volumes of alcohol and was purified and obtained free from nitrogen-containing bacterial debris, ash, etc., by fractional precipitation from aqueous solution by addition of various solvents.

A typical ash-free sample had $[\alpha]_D^{20°}$ − 45° (*c*, 1·0 in water) and could not be separated into fractions having different properties.

It was very readily hydrolysed by acids. Thus on being kept at 50° with N/10-sulphuric acid the specific rotation changed from $[\alpha]_D^{20°}$ − 43° to $[\alpha]_D^{20°}$ − 90° (equilibrium value) in 55 minutes. After removal of the acid the hydrolysate gave crystalline fructose in good yield, and determination of ketose sugar (Nyn, *Chem. Abs.*, 1925, **19**, 1236) showed that only fructose residues were present in the polysaccharide.

Methylation.—The procedure adopted was a slight modification of that described by Challinor, Haworth, and Hirst (*loc. cit.*) followed by treatment with silver oxide and methyl iodide. The product was a fine white powder soluble in acetone and chloroform and insoluble in ligroin, and was separated into fractions by use of these solvents. The main bulk of the methylated polysaccharide (*ca.* 60% yield) had OMe, 44·0%, $[\alpha]_D^{18°}$ − 56° (*c*, 0·5 in chloroform) (cf. Lyne, Peat, and Stacey, *loc. cit.*). It could not be separated into further fractions and so was used for the chain-length estimation.

Chain-length Estimation.—The various samples from the attempted fractionation were hydrolysed separately with oxalic acid in aqueous methanol. In a typical experiment the methylated polysaccharide (0·87 g.) was dissolved in methanol (30 c.c.), and oxalic acid (0·4 g.) in water (10 c.c.) added. On being

kept at 80° the rotation $[\alpha]_D^{29°} - 40°$ changed to $[\alpha]_D - 20°$ (equilibrium value) in 12 hours. The solution was neutralised with barium carbonate and filtered, and the solvent distilled off. By being kept in methanolic hydrogen chloride (100 c.c. of 0·25%), the residual sugar (0·85 g.) was converted into the methylglycosides, which were fractionally distilled in a high vacuum. The distillation behaved very similarly to that described previously for the methylated derivatives of the *Bact. pruni* levan (Lyne, Peat, and Stacey, *loc. cit.*). In the initial fractions (b. p. 80°/0·04 mm., $n_D^{18°}$ 1·4455, OMe, 58·0%) no furfuraldehyde or unsaturated decomposition products were detected. The percentage of "tetra" was estimated from the methoxyl contents of the fractions, for it was considered that the possibility of the occurrence of mixed pyranose and furanose forms made less reliable the result of calculations from the refractive indices of the fractions. Allowing a 10% correction for losses it was found that 3·00 g. of methylated levan yielded 0·265 g. of "tetra", *i.e.*, *ca.* 9% yield, which amount corresponds to a chain length of about 12 units. The tetra- and tri-methyl fructosides were identified as 1 : 3 : 4 : 6-tetra- and 1 : 3 : 4-tri-methyl fructofuranose respectively by the methods previously described (Challinor, Haworth, and Hirst, *loc. cit.*).

Attempted Quantitative Separation of Tetra- and Tri-methyl Fructose by Chromatographic Methods.—A large number of experiments were carried out in this unsuccessful work, but only some typical findings are herein reported. For most of the attempts the hydrolysate (OMe, 4·38%) from a sample of trimethyl inulin (OMe, 44·0%) served as the mixture of tetra- and tri-methyl fructoses. By a simple carbon tetrachloride–water partition method it was possible readily and consistently to separate a fraction in 10% of the mixture having OMe, 49·0% (Calc. for tetramethyl fructose: OMe, 52·0%). For further separation of the fractions (0·35 g.) by the use of essentially the column as described by Gordon, Martin, and Synge (*Biochem. J.*, 1941, **35**, 1338; 1943, **37**, 80; Gilbert, Smith, and Stacey, *J.*, 1946, 622) with carbon tetrachloride as eluting agent the following typical result was achieved:

Fraction.	Column-lengths of solvent required.	Wt. of fraction (g.).	OMe (%).
1	13 of carbon tetrachloride + 1 of 2% butanol in carbon tetrachloride	0·107	37·9
2	1 of 2·5% butanol in carbon tetrachloride	0·085	44·7
3	2 of 2·5% butanol in carbon tetrachloride	0·095	50·6
4	Extruded column extracted with boiling acetone (50 c.c.)	0·034	45·6
	Recovery	0·321	

In further experiments various types of continuous extraction devices were employed, and from the inulin hydrolysate a range of fractions having OMe, 19·8—47·2% were directly obtained, but in no case so far has a sharp quantitative separation been achieved.

Paper-strip Chromatography (with Dr. P. W. Kent).—The paper-chromatogram technique for the qualitative separation of reducing sugars (Partridge, *Nature*, 1946, **158**, 270) has been extended for qualitative separation of mixtures of partly methylated sugars. By using strips of filter paper (10 × 25 cm.) and butanol–water or butanol–acetic acid as the eluting agents, it was shown that a mixture of 2 : 3-dimethyl, 2 : 3 : 4-trimethyl, and 2 : 3 : 4 : 6-tetramethyl glucose could be readily separated. No separation of mixtures of 2 : 3 : 4-, 2 : 3 : 6-, and 3 : 4 : 6-trimethyl glucose could, however, be achieved. There was some separation of trimethyl glucoses from trimethyl mannoses and of trimethyl hexoses from trimethyl pentoses (cf. Flood, Hirst, and Jones, *Nature*, 1947, **160**, 86).

Mixtures of partly methylated sugars obtained by hydrolysis of methylated polysaccharides were examined by the technique, and by this means it was possible to identify a number of the constituents. Thus the hydrolysis mixture from the methylated *Pseudomonas mors-prunorum* levan (described above) showed two constituents only corresponding in R_F value (Partridge, *loc. cit.*) to 1 : 3 : 4 : 6-tetra- and 1 : 3 : 4-tri-methyl fructofuranose.

The University, Edgbaston, Birmingham 15. [*Received, October 20th*, 1947.]

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315. *Some Physical Properties of the Specific Polysaccharides from the Types I, II, and III Pneumococcus.*

By B. R. Record and M. Stacey.

Type I, II, and III pneumococcus polysaccharides have been obtained by the methods devised by Heidelberger and his colleagues (for references see Boyd, "Fundamentals of Immunology", Interscience, 1942) in relatively undegraded forms, and some of their general properties are described.

Ultracentrifuge and diffusion measurements have been made on the polysaccharides from the three types in order to determine their molecular weights and to gain knowledge of their molecular homogeneity and shape. The specimens showed deviations of considerable magnitude from the van't Hoff ideal, necessitating the extrapolation of both sedimentation and diffusion constants to infinite dilution in order to obtain a valid estimate of molecular weight. All three polysaccharides are polydisperse and consist of molecules of greatly extended shape.

A good deal of knowledge is now available from American investigations on the general chemical properties of several of the type specific polysaccharides of the pneumococcus. In one case, that of Type III, the chain structure of the repeating unit has been determined in some detail (Reeves and Goebel, *J. Biol. Chem.*, 1941, **139**, 511). Some ten years ago, one of us (M. S.) had the

opportunity of preparing and purifying some of the pneumococcus type specific polysaccharides in Professor M. Heidelberger's laboratory at Columbia University Medical School, while the other (B. R. R.) was able to examine these preparations in Professor Svedberg's laboratory at Uppsala. Circumstances beyond our control have delayed publication of these results until now.

Each type of polysaccharide was obtained from a virulent 24-hour culture which was grown, as described in the experimental section, in liquid medium containing, as essentials, glucose, phosphate, and either peptone or meat infusion broth. The polysaccharide was thrown out of the sterilised, filtered, and concentrated solution by means of alcohol. It was separated from extraneous protein material by being shaken for short periods in aqueous solution with chloroform and butanol, and was purified by fractional precipitation methods. Finally it was obtained in the form of the sodium salt which was a white fibrous powder having typical properties as shown in Table I for the Types I, II, and III material.

TABLE I.

Specific pneumococcus polysaccharide.	$[\alpha]_D^{20°}$ (sodium salt).	Equiv. (free acid).	Ash, %.	N, %.	P, %.	$\eta_{sp.}^{18°}$ (in water).	$\eta_{sp.}^{18°}$ (in 1% sodium chloride).
Type I	+230°	460	5·8	5·6	0·12	13·8	2·9
Type II	+ 59	740	3·8	0·34	0·18	12·8	2·5
Type III	− 36	340	5·9	0·35	0·15	18·8	4·1

It will be noted that some of these properties differ from those recorded by previous authors (see Brown, *J. Immunol.*, 1939, **37**, 445). In the case of both Types I and II polysaccharides the optical rotation was definitely lower than had previously been recorded. Despite many attempts at purification, avoiding strong reagents, it was not possible to increase the numerical value of the specific rotation of either polysaccharide. In the case of the Types II and III polysaccharides it was observed that every fraction contained a low but significant nitrogen and phosphorus content. It is considered that these elements are combined in a prosthetic group forming a firmly bound integral part of the macromolecule. Treatment of the viscous solution of the polysaccharide with alkali rapidly caused a degradation of the molecule as evidenced by an irreversible fall in relative viscosity and liberation of an insoluble flocculent nitrogen-containing fragment. It appears that the integrity of the macromolecule in each polysaccharide depended upon the presence of the firmly bound prosthetic group.

Ultracentrifuge and Diffusion Measurements.—In an investigation of the osmotic pressure-concentration relationship of a wide variety of polysaccharide derivatives in organic solvents (Carter and Record, *J.*, 1939, 660), it was shown that deviation, often considerable, from the ideal solution laws frequently occurred. Anomalies of a similar kind are found in the sedimentation of long-chain polymers such as polystyrenes (Signer and Gross, *Helv. Chim. Acta.*, 1934, **17**, 59, 335, 726); cellulose derivatives (Signer and Tavel, *ibid.*, 1938, **21**, 535; Kraemer, *Ind. Eng. Chem.*, 1938, **30**, 1200; Gralen, Dissertation, Uppsala, 1944; Jullander, *Arkiv Kemi, Min. Geol.*, 1945, **21***A*, No. 8). These deviations are manifest in a decrease in the sedimentation rate with increasing concentration, necessitating extrapolation to zero concentration, where the molecules no longer interfere with each other, in order to obtain a true value for the sedimentation constant of the free molecules. In diffusion also, the variation in the rate of diffusion with concentration leads to skew distribution curves in all but the most dilute solutions, and special precautions are necessary in deducing the true diffusion constant under conditions which permit free diffusion of the molecules. (See, *e.g.*, diffusion experiments on methylated and acetylated cellulose by Polson, *Kolloid Z.*, 1938, **83**, 172.) Methylated glycogen exhibits such anomalous effects only to a minor extent (see Record, this vol., p. 1567) evidently possessing a shape not far removed from the spherical. The capsular polysaccharides prepared from the pneumococcus, on the other hand, exhibit considerable deviations from the ideal behaviour in both sedimentation and diffusion. This section of the paper is concerned with the extrapolation of these two properties to zero concentration and the calculation of molecular weights and frictional ratios from the data so obtained for each of the three polysaccharides.

Methods and Results.—The methods employed follow precisely those described in the work on methylated glycogen (Record, *loc. cit.*). The polysaccharides prepared as described in the first part of this paper were stored in a vacuum over phosphic oxide. The dried specimens dissolve slowly in dilute salt solutions to form viscous solutions. Since they contain small quantities of sodium and are probably sodium salts, care must be taken to make up the solutions

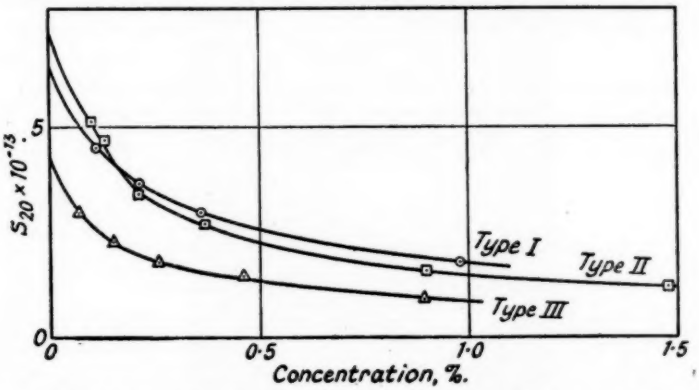
in sodium chloride of sufficient concentration to suppress any Donnan effects. With the exception of a few measurements in 0·5M-sodium chloride to check this point, 0·15M-sodium chloride was used throughout the present work and was found adequate in sedimentation and diffusion with the concentrations employed.

Sedimentation Velocity.—All runs were made in the Svedberg oil turbine centrifuge at the maximum routine speed of 70,000 r.p.m. (centrifugal field = 360,000 × gravity). A preliminary run on a 1% solution of the Type I polysaccharide showed a single and extremely sharp sedimentation boundary. The boundary showed no tendency to broaden as the run progressed, and indeed appeared as sharp at the end of the run (4 hours duration) as at the beginning. This behaviour suggested, at first sight, a substance possessing a high degree of molecular homogeneity and a low diffusion constant. On repeating the run on a 0·1% solution, however, not only did the boundary show a considerable broadening with time, but the sedimentation took place more than twice as rapidly. It is evident that this anomalous behaviour is the result of considerable departure from the ideal solution laws and that, if the sedimentation constant is to be used for the calculation of molecular weight, investigation of the sedimentation–concentration relationship and extrapolation to infinite dilution become essential.

The sedimentation constant determined at intervals during a run shows a tendency to increase as the run progresses, owing to a progressive dilution of the solution resulting from the combined effects of (*a*) the increase in the centrifugal field with increasing distance from the

FIG. 1.

Pneumococcus polysaccharides. Sedimentation–concentration curves.



axis of rotation, and (*b*) the sector shape of the cell. Owing to the difficulty of obtaining a well-defined drift in the calculated sedimentation constant during a run, a mean value has been taken over the duration of each run. This value refers strictly to a solution of slightly lower concentration than that placed in the cell at the commencement of the run. The error has been ignored in the present work since it has a negligible effect on the extrapolated value at infinite dilution. The sedimentation constants thus obtained, corrected to a medium of water at 20°, are given in Table II, together with the extrapolated values, $c \longrightarrow 0$. The general form of the sedimentation–concentration curves is shown in Fig. 1. The limiting values of s ($c \longrightarrow 0$) may be obtained from these curves, but can be extrapolated more conveniently from the 1/sedimentation–concentration curves which approximate more closely to straight lines.

One result of the large variation in the sedimentation constant with concentration has just been discussed, *viz.*, a tendency of the calculated s values to increase during a run owing to progressive dilution. Another consequence of this variation is seen in the exceptionally sharp sedimentation boundaries in all but the most dilute solutions. The smaller molecules which tend to lag behind the main boundary find themselves in a region of low concentration where their sedimentation velocity is greatly increased, while the larger particles sedimenting in a region of high concentration will have a relatively small sedimentation velocity; the net effect is an artificial sharpening of the sedimentation boundary. This effect calls for caution in deducing information about the degree of polydispersity from the rate of boundary spreading.

In many of the runs the boundaries were so sharp as to involve the deviation of only one of the scale divisions, representing only about 1/100 part of the distance down the centrifuge cell. In cases of this kind the diagonal Schlieren method of Philpot (*Nature*, 1938, **141**, 283), which

TABLE II.

Sedimentation constants (corrected to water at 20°) of pneumococcus polysaccharides in 0·15M-sodium chloride.

Type I.		Type II.		Type III.	
Concn., g./100 g. of 0·15M-NaCl.	$s_{20} \times 10^{13}$.	Concn., g./100 g. of 0·15M-NaCl.	$s_{20} \times 10^{13}$.	Concn., g./100 g. of 0·15M-NaCl.	$s_{20} \times 10^{13}$.
0·114	4·43	1·475	1·17	0·917 *	1·20
0·209	3·59	0·904	1·56	0·890	1·056
0·355	2·98	0·366	2·80	0·447	1·53
0·98	1·88	0·210	3·47	0·270	1·88
Limit $(c \longrightarrow 0) s_{20} = 6{\cdot}5 \times 10^{-13}$		0·126	4·48	0·158	2·40
		0·084	5·11	0·089	2·95
		1·390 *	1·30	Limit $(c \longrightarrow 0) s_{20} = 4{\cdot}3 \times 10^{-13}$	
		0·504 †	2·26		
		Limit $(c \longrightarrow 0) s_{20} = 7{\cdot}2 \times 10^{-13}$			

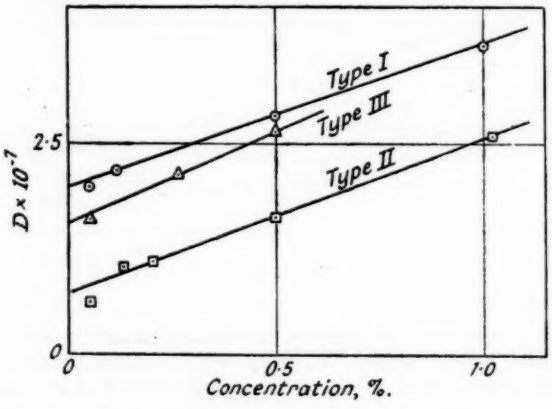
* These runs were made in 0·5M-NaCl.

† Run carried out at the Lister Institute using the Diagonal Schlieren Optical System (see Fig. 2).

was developed after most of the present work had been carried out, has advantages over the scale method, as it permits a more accurate determination of the boundary position and also of the degree of boundary spreading. The untouched photograph in Fig. 2 was obtained by means of this optical system installed by Dr. A. S. McFarlane on the oil-turbine centrifuge at the Lister Institute, London. It represents the sedimentation diagram for a 0·5% solution of the Type II polysaccharide in which the sharpening effect is most marked, but which is characteristic of all the three polysaccharides examined.

FIG. 3.

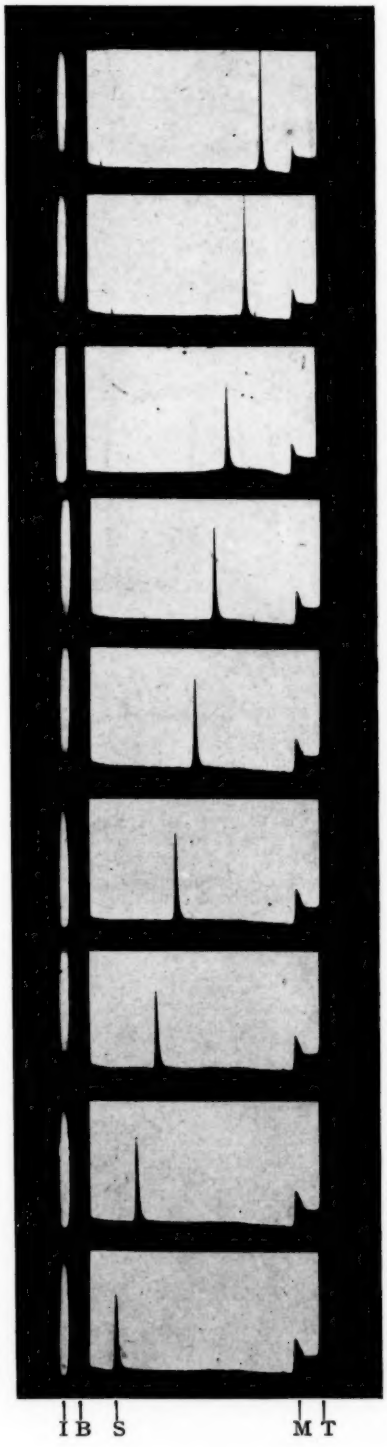
Pneumococcus polysaccharides. Diffusion–concentration relationship.



Diffusion.—The large increase in the sedimentation constant with diminishing concentration is in a direction corresponding to an apparent increase in the " effective " molecular weight of the particles on dilution. A similar effect in the same direction is found in diffusion. Thus when a 0·5% solution of the Type I polysaccharide in 0·2M-sodium chloride was allowed to diffuse against 0·2M-sodium chloride, an asymmetric concentration distribution was observed. Diffusion took place much more rapidly in the solution than in the solvent, so that the concentration gradient after a given time was steeper on the solvent side than on the solution side of the boundary. In order to follow the variation in the diffusion constant with concentration and at the same time avoid the difficulty of dealing with skew concentration distributions, one solution was allowed to diffuse against another of slightly different concentration, the concentration difference being taken as small as possible compatible with accurate definition of the concentration distribution. With differences of not more than 0·2%, practically symmetrical curves were obtained. The curves were analysed by the moment method by calculating the standard deviation for the whole curve from the first and second moments about the centroidal vertical. The measurements were made in the Lamm cell at 20°, and the calculated diffusion constants corrected to diffusion in pure water. The results are shown in Table III. The diffusion constants show a considerable increase with increasing

FIG. 2.

Sedimentation velocity diagram.



Pneumococcus Type II Polysaccharide.

0·5% *Solution in* 0·2M-NaCl.

Diagonal Schlieren Optical System.

Speed = 60,000 r.p.m.

Centrifugal Force = 250,000 × g.

Interval between Exposures: 30 mins.

Cell Magnification: 2·03.

Abscissæ: Distance from centre of rotor (x).

Ordinates: Concentration gradient (dc/dx).

T = *top of cell.*
M = *meniscus.*
S = *sedimentation boundary.*
B = *bottom of cell.*
I = *cell index.*

[*To face p.* 1564.

concentration and the relationship appears to be linear over the range examined (Fig. 3). The extrapolated values of D_{20} ($c \longrightarrow 0$) are given in the table.

The Partial Specific Volume.—This was determined at 20° for the Type I polysaccharide only. The values obtained were $V = 0{\cdot}538$ for a 1% solution and $V = 0{\cdot}536$ for a 0·5% solution. A mean value $V = 0{\cdot}537$ was used for all three polysaccharides.

The Molecular Weight.—The molecular weights of the three polysaccharides have been calculated from the Svedberg formula $M = \frac{RTs}{(1 - V\rho)D}$ using the above values of s and D extrapolated to infinite dilution. The results are shown in Table IV.

The Frictional Ratio.—The value of f/f_0, of between 3·2 and 6·0 (see Table IV) points to considerable asymmetry in the shape of the molecules, a conclusion in conformity with their observed properties, *e.g.*, high viscosity and large deviations from the ideal solution laws as seen in their behaviour in sedimentation and diffusion. On the assumption that there is no appreciable hydration of the particles, the actual values of the axial ratios of each of the polysaccharides have been deduced from the values of the frictional ratio using the equation of Perrin as described by Svedberg and Pedersen (" The Ultracentrifuge ", p. 41), though, as there pointed out, such figures may not have precise significance and are to be treated with reserve.

TABLE III.

Diffusion of pneumococcus polysaccharides in 0·2M-NaCl.

Type I.

Concn., g./100 g. of 0·2M-NaCl.	Mean concn.	Time, hours.	$D_{20} \times 10^7$.	Mean $D_{20} \times 10^7$.
0·10	0·05	36	1·87	2·02
0·10	0·05	35¼	2·16	
0·226	0·113	24	2·29	2·22
		36	2·14	
0·401	0·50	23	2·99	2·84
0·604		34	2·68	
0·53 *	0·26	52½	—	2·19
1·104	1·00	13¼	3·89	3·67
0·905		24½	3·46	

Limit D_{20} ($s \longrightarrow 0$) = $2{\cdot}0 \times 10^{-7}$

* Asymmetrical curve.

Type II.

0·100	0·05	23½	0·749	0·73
		35½	0·712	
0·102	0·195	21	1·104	1·10
0·287		32	1·096	
0·406	0·495	23	1·829	1·65
0·584		35	1·668	
		47½	1·451	
0·85	1·01	22	2·490	2·56
1·17		36	2·633	
0·069	0·122	16	1·241	1·06
0·175		28½	1·103	
		41	0·826	

Limit D_{20} ($s \longrightarrow 0$) = $0{\cdot}75 \times 10^{-7}$

Type III.

0·145	0·220	21½	2·386	2·18
0·295		33½	1·964	
0·390	0·501	23½	2·543	2·66
0·613		35½	2·773	
0·105	0·052	12	1·727	1·63
		23	1·533	
		36	1·624	

Limit D_{20} ($s \longrightarrow 0$) = $1{\cdot}60 \times 10^{-7}$.

TABLE IV.

Molecular weight and frictional ratio of pneumococcus polysaccharides.

Type.	$s_{20} \times 10^{13}$ ($c \longrightarrow 0$).	$D_{20} \times 10^7$ ($c \longrightarrow 0$).	M.	f/f_0.	Axial ratio b/a.
I	6·5	2·00	171,000	3·2	60
II	7·2	0·75	504,000	6·0	200
III	4·3	1·60	141,000	4·3	110

The Degree of Polydispersity.—As already pointed out, the sharpness of the sedimentation boundary in concentrations of 0·5% or more (see Fig. 2) may lead one to conclude that the three pneumococcus polysaccharides examined possess a high degree of molecular homogeneity. Bearing in mind their low diffusion constants, however, the boundary spreading observed at the lowest concentrations examined, *ca.* 0·1%, leaves no doubt as to the polydisperse nature of all three polysaccharides. Owing to the practical difficulties in the way of carrying out sedimentation-velocity runs in a sufficiently dilute region where mutual interference between the molecules is no longer significant, no attempt has been made to assess the degree of polydispersity. For a similar reason the equilibrium centrifuge cannot be directly applied to determine the molecular weight of such asymmetric molecules. Further consideration of the results of this paper suggests that even in the absence of any boundary sharpening effect, *i.e.*, in solutions sufficiently dilute to reduce the sharpening to negligible proportions, it may yet be possible to have polydispersity without boundary spreading. Thus, the Types I and II polysaccharides, although of widely different molecular weight, have almost identical sedimentation–concentration curves, so that a mixture of these two substances would sediment as one component. In such a case, the differences in molecular weight will be revealed almost entirely by differences in the diffusion constant, and with long-chain molecules this may prove to be a far more sensitive index of polydispersity than sedimentation velocity. In general, therefore, the behaviour in both sedimentation and diffusion must be taken into account in assessing the extent of the polydispersity.

How far the values quoted in Table IV for the molecular weight and shape of the three polysaccharides are truly representative of intrinsic differences between them and how far the result of differences in their treatment, purification, etc., can be decided only by extended investigation. It is at least clear, however, that the pneumococcus polysaccharide consists of large molecules with average molecular weights of the order 10^5—10^6 possessing greatly extended length in relation to their breadth.

Experimental.

Preparation and Properties of the Polysaccharides.—Tap water (20 l.) was heated to 80°; "Difco" Bacto-peptone (20 g. per litre) was added, the liquid boiled for about 10 minutes and cooled, and the pH adjusted to 7·8 by the addition of 3—3·5 c.c. of 2N-sodium hydroxide per l.

The filtered liquid was distributed in 1400 c.c. amounts in sterile two-litre Erlenmeyer flasks. A "Swift–Hodge" buffer solution was made up as follows: glucose, 5 g.; sodium bicarbonate, 2 g.; sodium chloride, 2 g.; hydrated disodium hydrogen phosphate, 1 g.; all dissolved in 1 l. of distilled water.

The phosphate was dissolved first and then the other salts to make 2 l. of solution which was sterilised by filtration through Chamberland L.2 or L.3 candles into sterile flasks. The broth solution was sterilised in an autoclave at 15 lbs. pressure for 15 minutes. To each flask was added the buffer solution (100 c.c.) under sterile conditions. The flasks were incubated at 37° for a few days in order to ascertain whether they were completely sterile. In the meanwhile a Type I pneumococcus organism was put through a "mouse passage" in order to increase its virulence.

After incubation overnight the tubes showed a healthy growth, and a loopful of the organisms was examined by the Gram- and capsule-staining methods. Sterile meat broth (300 c.c.) in 3 flasks was inoculated with 1 c.c. of the live suspension from the tubes and incubated overnight. There was a good growth, and this was used for inoculating the 20 l. of medium, each flask being inoculated with 10 c.c. of the prepared virulent culture of pneumococcus Type I and then incubated at 37° for 72 hours. Each flask showed a thick turbidity and a heavy deposit of the bacterial cells. The organisms were killed by the addition of phenol (10 c.c. of a solution of 10 g. per l.). After 3 hours, agar plates were made of the organism; later inspection showed that 3 hours with phenol was sufficient to kill all the organisms. The flasks were left for 24 hours before the contents were emptied. After being well mixed the solution was evaporated at room temperature in a vacuum. The volume of the concentrate was made to 2·75 l. with the washings, and in this syrupy liquid was dissolved 200 g. of sodium acetate and 20 c.c. of gacial acetic acid. The polysaccharide was precipitated as a white curdy mass by the addition of 95% ethanol (2·75 l.). After standing overnight the clear yellow supernatant liquid was siphoned off. The polysaccharide was isolated on the centrifuge. Previous experiments in Professor M. Heidelberger's laboratory showed that this polysaccharide material was very impure but that it probably contained about 1 g. of pure Type I polysaccharide.

Rapid Method for the Purification of the Type I Polysaccharide.—The precipitate was dissolved in distilled water (500 c.c.), forming a white emulsion. This was shaken on the mechanical shaker for 2 hours with chloroform (100 c.c.) and butanol (20 c.c.). This treatment rendered a large amount of the protein material insoluble, and this could be removed by 2 hours' centrifuging at 2000 revs. per minute. The precipitate was washed thrice by shaking it for ½ hour with water (50 c.c.), and the washings added to the polysaccharide solution (650 c.c.).

This liquid was shaken for 1 hour with chloroform (50 c.c.) and butanol (10 c.c.), centrifuged, and the precipitate washed as before with water (50 c.c.).

Sodium acetate (100 g.) and glacial acetic acid (20 c.c.) were dissolved in the solution and washings (total vol., 700 c.c.). The polysaccharide was precipitated by the cautious addition of 95% alcohol (700 c.c.). It was isolated on the centrifuge as before and the supernatant liquid rejected.

The polysaccharide was dissolved in water (300 c.c.) and shaken for 1 hour with chloroform (25 c.c.) and butanol (5 c.c.). After this solution had been centrifuged for 2 hours at 2000 revs. per minute there was a trace only of precipitate showing that all the protein had been removed. The polysaccharide was isolated as before by precipitation from acid solution, in the presence of sodium acetate, by an equal volume of alcohol. It formed a flocculent white precipitate which was free from glycogen and phosphate and gave no biuret test. It reacted very strongly in extremely dilute neutral solution, *e.g.*, in a dilution of 1 in 3 millions, with a suitable absorbed Type I pneumococcal antisera. It formed frothy opalescent solutions in water.

The polysaccharide was separated into fractions by precipitation from a solution containing 2% of sodium acetate with solvents such as acetone or alcohol. The material imparting the opalescence was first separated at low alcohol concentration and the main bulk of the polysaccharide (2·2 g. from 24 l. of culture medium) was obtained in the form of a white fibrous solid which could not be separated into further fractions by precipitation methods or by adsorption on kieselguhr, etc. {Found, for a typical preparation of the sodium salt: $[\alpha]_D^{20°}$ + 230° (*c*, 0·6 in water); $\eta_{sp.}^{18°}$ in water, 13·8; $\eta_{sp.}^{18°}$ in 1% sodium chloride, 2·9; N, 5·6; P, 0·12; ash (sodium), 5·8%; equiv. (free acid), 406}.

Products with similar properties and in similar yields were obtained by growing the pneumococci on a medium containing meat infusion broth instead of peptone. Products made on the medium were more readily separated from extraneous protein material.

Type II Specific Polysaccharide.—This was grown in a manner identical with that described for the Type I material. The polysaccharide was more readily obtained in homogeneous form, and yields were slightly higher, *e.g.*, 2·6 g. from 24 l. of medium. The product was a white fibrous solid, completely water-soluble {Found, for a typical preparation of the sodium salt: $[\alpha]_D^{20°}$ + 59° (*c*, 0·5 in water); $\eta_{sp.}^{18°}$ in water, 12·8; $\eta_{sp.}^{18°}$ in 1% sodium chloride, 2·5; N, 0·34; P, 0·18; ash (sodium), 3·8% equiv., (free acid), 740}. From an acid hydrolysate of this polysaccharide L-rhamnose was identified as a constituent unit.

Type III Polysaccharide.—This was produced on the above medium in the Birmingham laboratories from a Type III virulent pneumococcus culture provided by Dr. R. M. Jenner of Glaxo Laboratories Ltd. The product was a fibrous white solid which formed highly viscous solutions in water and in buffers, and it was difficult to purify {Found, for a typical sample of the sodium salt: $[\alpha]_D^{20°}$ − 36° (*c*, 0·4 in water); $\eta_{sp.}^{18°}$ in water, 18·8; $\eta_{sp.}^{18°}$ in 1% sodium chloride, 4·1; N, 0·35; P, 0·15; ash (sodium), 5·9%; equiv., (free acid), 390}.

Samples of closely similar preparations of the Type III polysaccharide were kindly provided by Dr. Elvin A. Kabat.

No claim is made that any of the preparations described above represent the pneumococcus polysaccharide in a completely undegraded state. All have been made from autolysed cultures, and it may yet be possible to extract from unautolysed cells polysaccharides with higher molecular weights than those described above.

The authors wish to thank Professor Sir Norman Haworth, F.R.S., for his keen interest in this work. One of them (B. R. R.) is deeply indebted to Professor The Svedberg for his hospitality at the Institute of Physical Chemistry, Uppsala, where the physical measurements were carried out with the assistance of a grant from the Department of Scientific and Industrial Research. The other (M. S.) expresses his warm thanks to Professor Michael Heidelberger for hospitality and generous co-operation in this work.

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316. *Ultracentrifugal and Diffusion Characteristics of Methylated Glycogen.*

By BASIL R. RECORD.

Ultracentrifuge and diffusion studies have been made on methylated glycogen from five different sources. The average molecular weights calculated from these data range from 1 to $4·4 \times 10^6$, in approximate agreement with osmotic-pressure estimates. All the specimens show considerable polydispersity, there being no particular molecular-weight species but a continuous range distributed more or less symmetrically about a mean value. No significant difference was observed between the methylated liver glycogen obtained from different sources or between methylated muscle and liver glycogen. The frictional ratio of 1·7—2·0 points either to appreciable hydration of the particles or to particles of a somewhat elongated form, or to a combination of both these effects.

OSMOTIC-PRESSURE measurements on methylated and acetylated polysaccharides (Carter and Record, *J. Soc. Chem. Ind.*, 1936, **55**, 218; *J.*, 1939, 660) have shown that in general their particle weights are many times greater than the weights of the basal chain units as determined by Haworth's end-group assay methods. Glycogen, both as the polysaccharide, and in the form of its methylated and acetylated derivatives (Oakley and Young, *Biochem. J.*, 1936, **30**, 868; Carter and Record, *loc. cit.*) appears to be outstanding in this respect, the estimated particle weights being upwards of 250 times the minimum molecular weights deduced from the end-group assay.

The methods developed by Svedberg based on sedimentation and diffusion measurements, besides providing an independent means of determining particle weights, contribute information on the degree of polydispersity, the presence of any well-defined molecular entities, and the extent to which the particles deviate from the spherical form.

The present work, which was carried out in Uppsala in 1938, is concerned with the application of such methods to the examination of methylated glycogen from a variety of sources.

Materials Examined.—Specimens of methylated glycogen prepared from the liver of the rabbit, hake, haddock, and dog-fish, and also a specimen from dog-fish muscle were available for investigation. The specimen of methylated rabbit-liver glycogen was prepared by Haworth, Hirst, and Isherwood (*J.*, 1937, 577). The Haworth end-group assay pointed to basal chain units of 18 glucose units. The remaining specimens were produced in a similar manner by Haworth, Hirst, and Smith (*J.*, 1939, 1914); they had similar methoxyl contents and consisted of " minimum molecules " of 12 glucose units. The specimens from rabbit liver and the livers of dog-fish, hake, and haddock have already been examined osmometrically (Carter and Record, *loc. cit.*).

The specimens were stored in a vacuum over phosphoric oxide.

Methods and Results.—The technique developed for the determination of sedimentation constants and molecular weights has been described in numerous publications by Svedberg (see, *e.g.*, *Ind. Eng. Chem.*, 1938, **10**, 113), and is dealt with in detail by Svedberg and Pedersen, (" The Ultracentrifuge ", Clarendon Press, 1940).

Concentration Gradients.—Throughout the present investigation these have been determined as refractive-index gradients by the method of Lamm (*Z. physikal. Chem.*, 1928, *A*, **138**, 313; 1929, *A*, **143**, 177; *Nova Acta Soc. Sci.*, 1937, Ser IV., **10**, No. 6) in which a scale is photographed through the solution. The deviation, Z, of the scale lines from their normal position on a reference scale is related to the refractive index gradient dn/dx by:

$$Z = G.\ a.\ b.\ dn/dx$$

where G = camera magnification of the scale; a = cell thickness;
b = scale distance from cell.

A mercury-vapour lamp was used as a light source and the scale photographs were recorded on ordinary process plates in conjunction with a suitable light filter, so that all measurements refer to a wave length $\lambda = 436$ mμ.

Sedimentation Velocity.—Sedimentation constants were determined in the oil-turbine centrifuge as described by Svedberg and Pedersen (*op. cit.*). The solutions were made up in 0·2M-sodium chloride to approximately 1% the day previously. The runs were made at a rotor speed of 25,000 r.p.m. (centrifugal force = 30,000 × gravity). The sedimentation diagrams, *i.e.*, concentration gradient plotted against x, the distance from the centre of rotation, showed only a single sedimenting boundary in each case. A considerable broadening of the boundary as the run progressed points to a high degree of polydispersity, since in view of the low diffusion constants (see below), almost all the spreading observed must be attributed to polydispersity in the specimens. Fig. 1 shows a sedimentation diagram typical of all the specimens examined.

Sedimentation Constants.—These were calculated from the rate of movement of the maxima of the concentration gradient curves, and corrected for the density and viscosity of the solvent to sedimentation in water at 20°.

The sedimentation constant $s_{20} = \frac{dx}{dt} \cdot \frac{1}{\omega^2 x} \cdot \frac{\eta}{\eta_0} \cdot \frac{1 - V\rho_0}{1 - V\rho}$ where $\frac{dx}{dt}$ = the sedimentation velocity observed, ω = the angular velocity, V = partial specific volume of the solute, η and ρ are the viscosity and density of the solution, η_0 and ρ_0 those of water at 20°.

The values obtained for the five specimens examined are given in Table I.

Diffusion.—In order to calculate molecular weight from sedimentation-velocity determinations, it is necessary to have an independent measurement of the diffusion constant. For this purpose a micro-diffusion cell of the Lamm type was used, with plane parallel windows and a bakelite slide for forming the boundary. Exposures were taken at suitable intervals after forming the boundary between solution and solvent, and a reference scale was obtained at the end of each experiment after mixing the contents of the cell.

In the case of substances with marked heterogeneity, the calculation of the diffusion constant at different points on the concentration-gradient curve gives a set of values which increase with distance from the original boundary. Therefore, in the present work the standard deviation,

σ, was calculated for the whole curve from the first and second moments about the centroidal vertical (Lamm and Polson, *Biochem. J.*, 1936, **30**, 528). The diffusion constant is given by

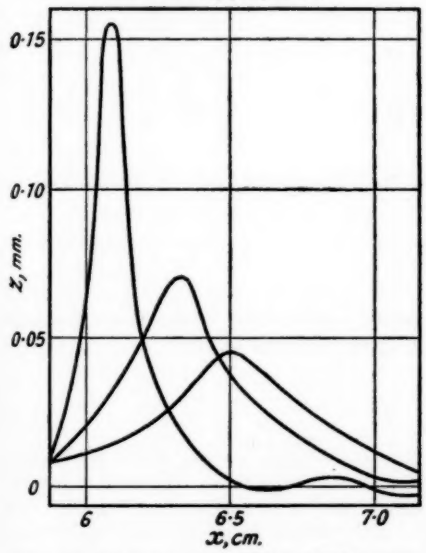
$$D = \frac{\left(\frac{1}{G}\right)^2 \sigma^2}{2t} \cdot \frac{(l-b)^2}{l^2}$$

where G = photographic enlargement factor of scale; t = time from formation of boundary; l = optical distance from scale to camera objective; b = optical distance from scale to centre of diffusion cell.

The solutions were made up to approximately 0·5% in 0·2M-sodium chloride and allowed to diffuse against 0·2M-sodium chloride. All measurements were made at 20·0° and corrected for the viscosity of the salt solution to diffusion in pure water. Fig. 2 shows a concentration gradient typical for the specimen examined.

FIG. 1.

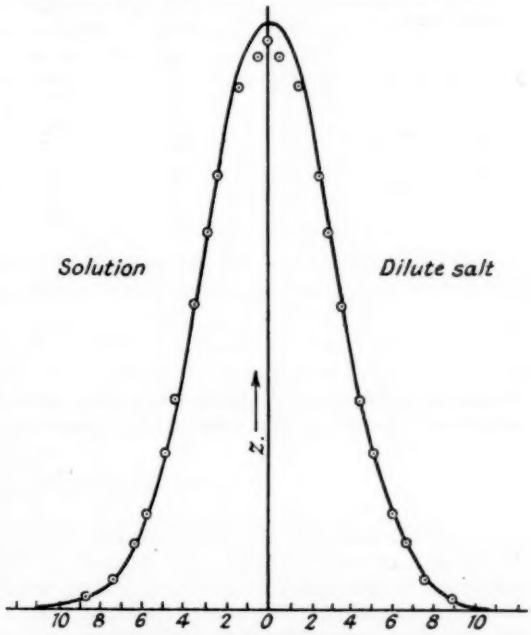
Sedimentation diagram of methylated haddock-liver glycogen (B).



c = 1·02%: *average speed* = 24,900 *r.p.m.*: *cell thickness* = 12 *mm.*: *scale distance* = 2·00 *cm.*: *interval between exposures* = 20 *minutes.*

FIG. 2.

Diffusion of methylated haddock-liver glycogen (B).



c = 0·99% *in* 0·2M-*sodium chloride. Exposure taken* 105½ *hours after forming boundary. Abscissæ represent vertical distances in the cell; ordinates are refractive-index gradients. The points marked refer to an ideal distribution curve having the same* σ *value as the experimental curve.*

In every case the curves were very nearly symmetrical, suggesting that the diffusion is independent of concentration in the range examined. This is in contrast to the abnormal behaviour found in the case of certain methyl and acetyl celluloses (Polson, *Kolloid Z.*, 1938, **83**, 172). The divergence from the ideal distribution curve having the same standard deviation is due to the polydisperse nature of the specimens. The apparent decrease in the diffusion constant with time (see Table I) may also be attributed to the presence of smaller more rapidly diffusing particles.

The Molecular Weight—The molecular weight of a substance can be calculated from its sedimentation and diffusion constants by means of the Svedberg formula (*Kolloid Z.*, Zsigmondy Festschrift, 1925, 37)

$$M = \frac{RTs}{D(1 - V\rho)}$$

where R = gas constant, T = absolute temperature, V = the partial specific volume of the solute, and ρ = the density of the solution.

The partial specific volume, V, was determined pyknometrically at 20° for an aqueous, electrolyte-free solution of methylated rabbit-liver glycogen which had been stored in an evacuated desiccator over phosphoric oxide for some months. The values 0·750 and 0·751 were obtained for a 2·12% and 1·05% solution respectively. The value 0·750 was assumed for the other specimens.

The molecular weights thus calculated for the five different specimens vary from $1·0 \times 10^6$ to $3·6 \times 10^6$ (see Table I).

TABLE I.

Methylated Glycogen. Collected Data.

Specimen.	$s_{20} \times 10^{13}$.	$D_{20} \times 10^7$. Time, hours.	D_{20}.	Mean.	$M_S \times 10^{-6}$.	f/f_0.	$M_E \times 10^{-6}$.
Rabbit liver	25·9	48	1·39	1·33	1·90	1·95	2·57
		72	1·27				
Dog-fish liver	41·2	55¼	1·14	1·12	3·58	1·87	4·40
		74¾	1·10				
Hake fish liver	20·7	59½	1·78	1·78	1·13	1·72	2·51
		36	1·86				
		49	1·67				
Haddock liver (*A*)	15·7	59	1·61	1·57	0·97	2·05	1·71
		85½	1·52				
Dog-fish muscle	34·6	48	1·47	1·35	2·50	1·75	—
		72	1·33				
		95	1·26				

The Frictional Ratio.—The molar frictional constant of a substance may be calculated from its sedimentation constant and molecular weight to be

$$f = \frac{M(1 - V\rho)}{s}$$

(Svedberg and Sjogren, *J. Amer. Chem. Soc.*, 1929, **51**, 3594). If particles of the same molecular weight and partial specific volume were spherical, then, from Stokes's formula

$$f_0 = 6\pi\eta N\left(\frac{3MV}{4\pi N}\right)^{1/3}$$

where η is the viscosity of the solvent and N the Avogadro constant. Thus, if the particles of a substance are non-solvated and spherical, the ratio f/f_0 should be unity. A value exceeding unity may be due either to solvation of the particles, considered spherical, or to deviation from the spherical shape. The nearly constant values (1·7—2·0) obtained for the five methylated glycogen specimens examined (Table I) suggest a similarity in general structure and are probably due to the combined effect of hydration and molecular asymmetry.

The Effect of Concentration on Sedimentation and Diffusion.—The molecular-weight data presented in Table I do not take into account the possibility of errors in the s and D values arising from deviations from the ideal solution laws. The s values quoted in Table I are those obtained for 1% solutions. 0·5% Solutions were used in the diffusion experiments, and there was no evidence, in the form of skewness in the distribution on either side of the boundary, of any concentration effect on diffusion in 0·5% solutions. The limited quantity of several of the specimens available precluded extended investigations of the effect of concentration in sedimentation and diffusion. Detailed measurements have, however, been made on an independent specimen of methylated haddock-liver glycogen (preparation *B*) which was available in larger quantity.

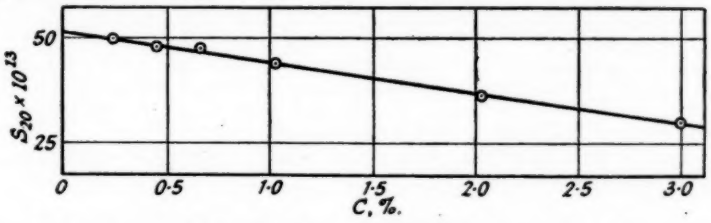
The variation in the sedimentation constant with concentration for this specimen in 0·2M-sodium chloride is shown in Fig. 3. A value obtained for a 2% solution in 0·5M-sodium chloride lies closely on the curve, showing that 0·2M-sodium chloride is sufficient to suppress any ionic effects. The value of s_{20} extrapolated to zero concentration is 51×10^{-13}. It is seen that the sedimentation constant decreases with increasing concentration, corresponding to an apparent decrease in the particle weight. A similar effect was observed in the osmotic pressure behaviour (Carter and Record, *loc. cit.*), the π/c values increasing with increasing concentration. The magnitude of the effect appears to depend on the extent to which

the particles deviate from the spherical shape. It is much greater in the polystyrenes (Signer and Gross, *Helv. Chim. Acta*, 1934, **17**, 59, 335, 726), various cellulose derivatives (see the recent comprehensive studies by Gralen, Dissertation, Uppsala 1944, and Jullander, *Arkiv Kemi, Min. Geol.*, 1945, **21***A*, No. 8), and the pneumococcus polysaccharides (this vol., p. 1561).

The effect of concentration on diffusion is shown in Table II. The diffusion constant for each exposure was calculated as before from the σ value obtained from the 1st and 2nd moments. The mean values obtained at three different concentrations show only variations inside the experimental error. The concentration-gradient curves were symmetrical in all cases (see, *e.g.*, Fig. 2). A fourth experiment in which a 1·17% solution was allowed to diffuse against one of 0·83% instead of 0·2M-sodium chloride gave a value in excellent agreement. In substances showing appreciable deviations from the van't Hoff law, asymmetrical curves are obtained, owing to the rate of diffusion on the solvent side of the boundary being different from that on the solution side. Such anomalies were observed in methylated cellulose (Polson, 1938; see also Graten, *loc. cit.*, and Jullander, *loc. cit.*) and the pneumococcus polysaccharides (Record and Stacey, *loc. cit.*).

FIG. 3.

Methylated haddock-liver glycogen (B) *: variation of sedimentation with concentration.*



The Error in calculating the Molecular Weight.—Taking a value of $1·03 \times 10^{-7}$ for D at zero concentration, we obtain for M_S a value of $4·82 \times 10^6$ and $f/f_0 = 1·84$. If the value of s_{20} for a 1% solution, *i.e.*, $43·0 \times 10^{-13}$, be taken instead of its extrapolated value, $M_S = 4·07 \times 10^6$ and $f/f_0 = 1·95$. Thus, owing to the variation in the sedimentation constant with concentration, the values of M_S given in Table I may be about 15% too low, and the true f/f_0 values will be slightly lower than those recorded.

TABLE II.

Effect of Concentration on Diffusion.

Methylated haddock-liver glycogen (*B*) in 0·2M-sodium chloride.

Concn., g./100 g. of solvent.	Time from start (hours).	$D_{20} \times 10^7$.	Mean $D \times 10^7$.
0·99	48	1·07	
	75	1·08	1·10
	105½	1·16	
0·517	48	1·05	
	72	0·99	1·03
	94	1·00	
0·192	48	1·06	
	70½	1·02	1·04
1·165 } 1·00	40	1·11	
0·830 }	68½	1·06	1·06
	88½	1·01	

Sedimentation Equilibrium Measurements.—The molecular weight of a solute can be calculated from the concentration distribution at equilibrium in a centrifugal field from the Svedberg formula :

$$M = \frac{2RT \log c_2/c_1}{(1 - V\rho) \cdot \omega^2(x_2^2 - x_1^2)}$$

where c_2 and c_1 are the concentrations of the dissolved substance at the distances x_2 and x_1 from the axis of rotation, and ω is the angular velocity. The solutions must be sufficiently dilute to avoid anomalies due to deviation from the van't Hoff law. The sedimentation and diffusion measurements on methylated haddock-liver glycogen above indicate that such deviations are of such a magnitude as not to involve serious errors in concentrations below 1%. Four of the

methylated glycogen specimens in concentrations of 0·5—1·0% in 0·2M-sodium chloride have been examined in the Svedberg equilibrium centrifuge. Equilibrium was reached after 7—10 days' centrifuging. The concentration distribution curve was deduced from the refractive index gradient curve by the procedure described by Pedersen (*Biochem. J.*, 1936, **30**, 948), and the molecular weight calculated over 0·05 cm. intervals down the cell. The specific refractive increment used in converting dn/dx to dc/dx was calculated from the area of the diffusion curves (see below), and was found to be fairly constant for all the specimens. A mean value of $13{\cdot}2 \times 10^{-4}$ ($\lambda = 436$ mμ) was used. The details of a typical equilibrium run are given here to show the variation in molecular weight with distance from the axis of rotation (Table III). The final result is expressed as a weight average.

TABLE III.

Equilibrium run on methylated dog-fish liver glycogen.

Concentration at start	= 0·767 g./100 g. of 0·2M-sodium chloride.
Thickness of cell used	= 6·03 mm.
Bottom of cell	= 5·92 cm. from axis of rotation.
Meniscus of solution	= 5·55 cm. ,, ,, ,, ,,
Optical scale distance	= 7·20 cm.
Scale magnification, G	= 1·047
Temperature	= 20·0°
Average speed	= 22·5 revs. per second.
Shape of cell	= Sector.

Equilibrium was reached after 9 days' centrifuging.
Exposures used in the calculation were taken 210½, 219, and 234 hours after the start.
The following analysis was obtained :

Distance from axis of rotation (cm.).	Concentration gradient, dc/dx.	Concn., g./100 g. of salt solution.	$M \times 10^{-6}$.
5·55	0·650	0·368	1·59
5·60	1·036	0·410	2·25
5·65	1·450	0·472	2·72
5·70	1·967	0·557	3·10
5·75	2·801	0·675	3·61
5·80	4·284	0·849	4·35
5·85	6·968	1·125	5·29
5·90	—	—	—

The weight average $M = 4{\cdot}40 \times 10^6$.

The molecular-weight analysis thus obtained does not necessarily represent the molecular-weight distribution of the specimen, and is to be regarded only as an indication of the range of particle weights present. The smooth concentration gradients obtained in the sedimentation-velocity experiments, however, point to the absence of any definite molecular species and to the existence of a continuous range of particle sizes.

The weight average values for the four specimens examined are given in Table I (M_E). These values tend to be somewhat higher than those obtained from sedimentation and diffusion measurements. The discrepancy may arise partly from the error in extrapolating the limits of the refractive index gradient curve in the sedimentation equilibrium experiments. The appropriate value for the refractive index increment to be used in converting the refractive index gradient curve into concentrations throughout the cell to obtain values comparable with the sedimentation–diffusion results is also open to some doubt. Pedersen (*Biochem. J.*, 1936, **30**, 948) has suggested that this increment is not that obtained by direct measurement or from the area of the diffusion curves, but more correctly that determined from the area of the curve in a sedimentation-velocity run, which in the present investigation is some 20—30% lower in the different specimens, suggesting the presence of material of higher or lower molecular weight than shown by the curves. The diffusion constants, however, refer to an average value for the total dissolved material, so that perfect agreement between the M_S and M_E values cannot be expected. Considerations of the question as to what kind of average values are being determined in the sedimentation and diffusion experiments may throw further light on the problem. This subject is dealt with at length in the recent treatises by Gralen and Jullander (*locc. cit.*).

The Degree of Polydispersity.—In substances with a high sedimentation, a rapid spread of the sedimentation boundary during a run is a fairly certain indication of considerable polydispersity, since the true diffusion constant of high molecular weight substances is correspondingly small.

A comparison of the apparent diffusion constant determined from the sedimentation–concentration gradient curves with the true diffusion constant is a useful indication of the presence and extent of the polydispersity. The apparent diffusion constants, or boundary spreading coefficients (MacFarlane, *Biochem. J.*, 1935, **29**, 407), have been calculated from the concentration gradients in sedimentation velocity runs on methylated haddock-liver glycogen (*B*) from the formula :

$$K = \frac{1}{4\pi(t_a - t_b)}\left[\frac{A_a^2}{Z_a^2} - \frac{A_b^2}{Z_b^2}\right]$$

where A_a, A_b are the areas and Z_a, Z_b the maximum heights of the curves at times t_a, t_b. The values of K appear to increase as the run progresses. K also decreases with increasing concentration. Calculations from the first two exposures (20 minutes interval) of each run give values of K ranging from 30×10^{-7} for a 3% solution up to 90×10^{-7} for a 0·22% solution, in contrast to the value of $1{\cdot}0 \times 10^{-7}$ for simple diffusion. It is evident therefore that the specimens are very polydisperse. The polydispersity of aqueous solutions of glycogen extracted from rabbit liver was mentioned by Mystkowski (*Biochem. J.*, 1937, **31**, 716), though in the absence of diffusion measurements there was no adequate evidence for this statement.

The Sedimentation Behaviour of Glycogen.—Sedimentation-velocity runs were carried out on two specimens of haddock-liver glycogen : (*a*) the crude polysaccharide once precipitated from water with alcohol; (*b*) the polysaccharide regenerated from glycogen acetate (see Haworth, Hirst, and Smith, *loc. cit.*). The solutions were made up to *ca.* 1% in 0·2M-sodium chloride. The concentration-gradient curves closely resembled those shown in Fig. 1, and the calculated sedimentation constants, 41·0 and 39·4 respectively, were of the same order. The partial specific volume of the crude glycogen specimen was 0·64, a value appreciably lower than that for methylated glycogen. However, the slower sedimentation consequent on the larger partial specific volume of the methylated polysaccharide is offset to some extent by the increase in the weight of the glycogen molecule due to the presence of methoxyl groups. This brief examination suggests that the average particle size of glycogen has a magnitude of the same order as its methylated derivative, and that acetylation and reconversion back to the polysaccharide has very little effect on the size of the physical units. This further substantiates the conclusions reached from the osmotic-pressure data (Carter and Record, *loc. cit.*) that the forces responsible for the aggregation of the basal chain units are of a more permanent nature than those occurring in ordinary physical aggregation, and lends support to the view expressed by Haworth, Hirst, and Smith (*loc. cit.*) that the union is effected by bonds of the primary valency type.

Discussion.—Sedimentation and diffusion measurements on methylated glycogen from five different sources point to average particle weights of $1{\cdot}0$—$4{\cdot}8 \times 10^6$. The slightly higher values obtained from sedimentation equilibrium measurements are in fair agreement when allowance is made for the errors introduced by the polydisperse nature of the material. The polydispersity has been demonstrated by comparing the rate of spread of the boundary during sedimentation with the spread resulting from diffusion only; spreading coefficients nearly 100 times larger than the diffusion constant are found for dilute solutions. This high degree of polydispersity appears to be characteristic of the polysaccharides, and is in marked contrast to the homogeneous nature of the proteins.

The above estimates of the average molecular weight of methylated glycogen are of the same order as those deduced from osmotic-pressure measurements, where values of $0{\cdot}3$—$0{\cdot}8 \times 10^6$ were obtained for the same specimens (Carter and Record, *loc. cit.*). The osmotic-pressure values are number averages and would therefore be expected to be of a lower order than those obtained from the centrifuge data for substances of such a polydisperse nature. Sedimentation runs on glycogen itself and on glycogen regenerated from the acetate provided sedimentation diagrams which are indistinguishable from those for the methylated derivative, suggesting that glycogen may be acetylated, methylated, and regenerated without any gross change in its particle size distribution. From osmotic-pressure measurements, Oakley and Young (*loc. cit.*) obtained values of $1{\cdot}1$—$2{\cdot}3 \times 10^6$ for rabbit-liver and muscle glycogen preparations which had been dialysed free from traces of impurities of low molecular weight; they were also able to show that methylation of the glycogen had very little effect on the mean particle weight, and that this was but little affected by the solvent, whether N/10-calcium chloride or benzene. Meyer and Jeanloz (*Helv. Chim. Acta*, 1943, **26**, 1784) have recorded a value as high as 6×10^6 for acetylated muscle glycogen from the mollusc, on the basis of osmotic-pressure measurements in benzyl alcohol. It seems probable that the variations in the particle weight of glycogen and its methylated and acetylated derivatives do not represent any intrinsic differences in the glycogen from different sources but are rather the result of differences in its extraction, treatment,

purification, etc. The large differences found between two specimens of methylated haddock-liver glycogen in the present work strongly suggest that this is the case.

The f/f_0 values (1·7—2·0) are substantially constant for all the specimens examined. If the departure from unity is the result solely of deviation from the spherical shape, it represents only a moderate degree of asymmetry; it is more likely, however, due to the combined effect of hydration and a relatively small degree of asymmetry. The relatively small effect of concentration on sedimentation and diffusion is in conformity with this view. Much higher asymmetry coefficients, together with large concentration effects, have been observed with cellulose derivatives and the pneumococcus polysaccharides.

The estimates of the particle weight of glycogen and its derivatives by various physico-chemical methods all point to physical units several hundred times greater than those deduced by the end-group assay method of Haworth. There is evidence that the chemical molecules are firmly linked together by primary valencies to form the large physical units. Considerations of shape lead one to conclude that the linkages do not take place only in an end-to-end direction, as in the long-chain molecules of cellulose, but also in breadth.

The author is indebted to Professor The Svedberg for the generous hospitality afforded at his Institute; also to Dr. K. O. Pedersen and others for help and advice. Grateful thanks are due to Professor Sir Norman Haworth, F.R.S., for his interest in this work and for the specimens examined. Acknowledgement is made to the Department of Scientific and Industrial Research for a grant.

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317. *Studies of the Autoxidation of Tetralin. Part I. Investigation of Autoxidation Products.*

By ALAN ROBERTSON and WILLIAM A. WATERS.

Several products have been isolated from the autoxidation of tetralin, the chief being α-tetralone and α-tetralol. Some of the minor products have been shown to be autoxidation products of α-tetralone, which itself forms a hydroperoxide that breaks down to a 1 : 2-diketone and then to acidic substances. *cyclo*Hexanone similarly gives *cyclo*hexanedione, and adipic acid and its hemi-aldehyde.

γ-*o*-Hydroxyphenylbutyric acid is also an autoxidation product of tetralin, and plays the important rôle of being an inhibitor of autoxidation. It can be made by oxidising α-tetralone with Caro's acid. An ionic mechanism is proposed for the formation of this substance, and, in general, for the per-acid oxidations of ketones.

IN an earlier paper (*Trans. Faraday Soc.*, 1946, **42**, 201) we pointed out that the chemical reactions concerned in the autoxidation of tetralin, and of its analogues (compare Waters, *Ann. Reports*, 1945, **42**, 130—143), can be divided into two consecutive phases, *viz.*, (I) formation of tetralin hydroperoxide, and (II) breakdown of the hydroperoxide, and showed that in the early stages of the autoxidation the *whole* of the oxygen uptake was due to the formation of tetralin hydroperoxide by a chain reaction involving free radicals. We have now found that the later stages of the autoxidation also involve the autoxidations of certain of the breakdown products of tetralin hydroperoxide, *e.g.*, α-tetralone. Some of these products too can be oxidised directly by tetralin hydroperoxide.

By studying separately the decomposition of pure tetralin hydroperoxide in the absence of fresh oxygen (see Part II, following paper) we have discriminated between direct and secondary oxidation processes sufficiently clearly to be able to interpret all earlier studies of the autoxidation on a much more comprehensive basis than has been possible hitherto (see Part III, this vol., p. 1585).

The Final Oxidation Products of Tetralin.—After about 80 hours of autoxidation at 76°, tetralin is converted into a viscous reddish-orange oil, containing *ca.* 25% of tetralin hydroperoxide; at an earlier stage, however, the peroxide content of the liquid is much higher (*ca.* 35—40%). Further oxygen uptake is thereafter very slow indeed.

After the remaining hydroperoxide has been destroyed by heat, the components of the resultant mixture can be separated by fractional distillation under reduced pressure, and in this way the following products have all been characterised: (1) 1 : 2-Dihydronaphthalene, comprising approximately 2% of the liquid, (2) α-tetralone, (3) α-tetralol, (4) γ-*o*-*hydroxyphenylbutaldehyde*, (5) γ-*o*-hydroxyphenylbutyric acid, (6) β-*o*-carboxyphenylpropionic acid, together

with polymeric products which could not be distilled. The lactone of (5) is probably present in the original mixture, whilst small amounts of the α-tetralyl esters of (5) and (6) may be formed during the distillation process, since the less volatile fractions of the oil contain small amounts of saponifiable substances. All these compounds have also been obtained by thermal decomposition of tetralin hydroperoxide in the absence of oxygen.

The acid (5) had been prepared from α-tetralone by oxidation with Caro's acid (Schroeter, D.R.-P. 562,827 of 1928). It is not formed by the autoxidation of α-tetralone (see below), whereas acid (6) is a major product of the autoxidation of α-tetralone. The production of both (5) and (6) indicates that α-tetralone can undergo further oxidation in two different ways. We regard (4) as a direct thermal decomposition product of tetralin hydroperoxide.

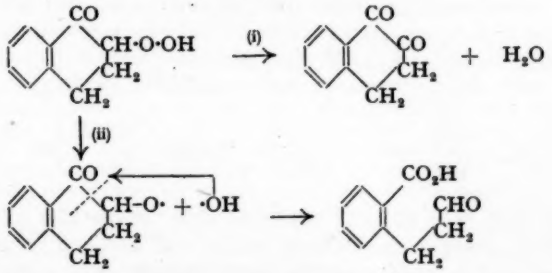
Autoxidations of Cyclic Ketones.—The foregoing work shows that the autoxidations of ketones and of hydrocarbons are interrelated. Previous studies of the autoxidation of ketones have not yielded clear information as to the nature of the autoxidation process. Jenkins (*J. Amer. Chem. Soc.*, **1935**, **57**, 2733) showed that ketones of the type $R{\cdot}CO{\cdot}CH_2R'$ can autoxidise, and give strong peroxide reactions after exposure to air. He suggested that the oxygen attacked their enolic forms as follows :

$$R{\cdot}CO{\cdot}CH_2{\cdot}R' \rightleftharpoons R{-}\underset{OH}{C}{=}CH{-}R' \longrightarrow R{-}\underset{O}{C}(OH){-}\underset{O}{C}HR' \longrightarrow R{\cdot}CO_2H + R'{\cdot}CHO$$

Paquot (*Bull. Soc. chim.*, **1945**, **12**, 450), however, obtained diketones, as well as aldehydes and acids, by autoxidation of a whole series of ketones in the presence of nickel phthalocyanine, and, in accordance with the theories of Farmer and his colleagues (*Trans. Faraday Soc.*, **1942**, **38**, 348; **1946**, **42**, 228), suggested that a carbonyl group activates an adjacent methylene group sufficiently to allow of the formation of a ketone hydroperoxide :

$$R{\cdot}CO{\cdot}CH_2{\cdot}R' \longrightarrow R{\cdot}CO{\cdot}\underset{O{\cdot}OH}{C}H{\cdot}R' \begin{cases} \longrightarrow R{\cdot}CO{\cdot}CO{\cdot}R' \\ \searrow R{\cdot}CHO + R'{\cdot}CHO \end{cases}$$

We have found that at 100° *cyclo*hexanone is easily autoxidised and soon gives positive peroxide tests with potassium iodide, and also an acid reaction to litmus. After 10 hours' treatment a heavy deposit of adipic acid settles out on cooling, whilst the residual liquid contains both *cyclo*hexane-1 : 2-dione and the hemi-aldehyde of adipic acid. β-Tetralone, which contains the $\cdot C_6H_4{\cdot}CH_2{\cdot}CO\cdot$ group is much more rapidly autoxidised than is α-tetralone : it yields β-*o*-carboxyphenylpropionic acid (6). α-Tetralone autoxidises *slowly* at 100°, yielding an orange-red liquid (compare tetralin itself) which gives a positive peroxide test and an acid reaction with litmus. The red liquid gave positive tests for a 1 : 2-diketone, and colour reactions with alkali indicative of β-naphthaquinol, which is a tautomeride of tetrahydronaphthalene-1 : 2-dione (compare Strauss, Bernouilly, and Mautner, *Annalen*, **1925**, **444**, 165). These results accord with Paquot's conclusions, and indicate that cyclic ketones form hydroperoxides which can either (i) dehydrate to 1 : 2-diketones or (ii) undergo a more complex decomposition which leads to a carbon–carbon bond fission similar to that suggested by Walsh (*Trans. Faraday Soc.*, 1946, **42**, 269) for the breakdown of tertiary hydroperoxides :



There is no indication, even with the hydroperoxide of β-tetralone, that the second of these oxidation processes affects the fission of the Aryl–C link.

The Rôles of Secondary Products in the Autoxidation of Tetralin.—(a) *α-Tetralone.* On the technical scale ketones (*e.g.*, methyl ethyl ketone) have been employed as promoters of the autoxidation of hydrocarbons (*e.g.*, pentane; U.S.P. 2,265,948 of 1939), and often do reduce

considerably the duration of the initial " induction period " in which there is very little oxygen uptake. On the other hand, Medvedev and Podyapolskaya (*J. Phys. Chem. Russia*, 1939, **12**, 719) found that the addition of α-tetralone reduced the overall rate of autoxidation of tetralin, and this has been confirmed by George and Robertson (*Proc. Roy. Soc.*, 1946, *A*, **185**, 309). Evidently the net rôle of a ketone as a promoter or as an inhibitor of autoxidation depends on (i) the relative ease of autoxidation of the hydrocarbon and the ketone concerned, and, more particularly, on (ii) the relative stabilities of their two hydroperoxides, which by their decompositions generate chain-starting hydroxyl radicals. Thus *cyclo*hexanone, which contains an active methylene group, is more easily autoxidised than *cyclo*hexane, whereas tetralin, which itself contains two active α-methylene groups, is more reactive than α-tetralone, and apparently yields a more stable hydroperoxide.

(b) *Phenolic products.* Nearly all phenols act as inhibitors in the initial stage of the autoxidation of hydrocarbons, though only certain easily oxidised ones, such as α-naphthol, are also effective once an appreciable amount of hydroperoxide has been formed (Robertson and Waters, *loc. cit.*). Bolland and ten Have (*Trans. Faraday Soc.*, 1947, **43**, 201) have shown that these typical inhibitors are themselves oxidised by chain-breaking processes. Now, the autoxidation rate of tetralin, in common with that of most naphthenic hydrocarbons (Larsen, Thorpe, and Armfield, *Ind. Eng. Chem.*, 1942, **34**, 182), begins to decrease when *ca.* 30% of the hydrocarbon has been oxidised, and becomes quite small at about the stage when, in phase (II) of the oxidation process, the phenolic secondary products (4) and (5) appear in appreciable amounts. The following table shows that γ-o-hydroxyphenylbutyric acid (5) is a marked inhibitor of the autoxidation of tetralin even if added at the stage of rapid oxygen uptake.

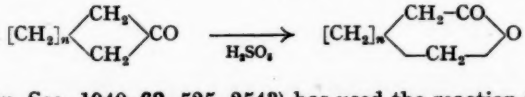
The effect of γ-o-hydroxyphenylbutyric acid on the autoxidation of tetralin at 76°.

	Oxygen uptake: ml. per 15 mins. Time after start of oxidation, mins.						
	15.	30.	45.	60.	75.	90.	105.
Partly oxidised tetralin	4·3	4·6	4·9	5·4	5·7	—	—
Same sample of tetralin plus 1 g. of acid (5) ...	2·2	1·9	1·7	1·4	1·0	0·9	0·5

Our studies of the minor products of the autoxidation of tetralin have thus afforded a rational explanation of even the subsidiary features of oxygen-uptake measurement.

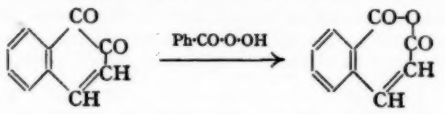
The Mechanism of Oxidation of Ketones with Hydroperoxides, including Caro's Acid.—Since γ-o-hydroxyphenylbutyric acid (5), one of the minor autoxidation products of tetralin, was not obtained by the autoxidation of α-tetralone, but can be obtained, in good yield by the action of Caro's acid, $HO{\cdot}O{\cdot}SO_3H$, which is of course a hydroperoxide, on α-tetralone, it probably results from the reaction between α-tetralone and tetralin hydroperoxide. If, as we believe (see Parts II and III) the thermal decomposition of tetralin hydroperoxide yields some free hydroxyl radicals, then it would be expected that it should behave as a hydrogen-abstracting, or as a hydroxylating agent, but it is difficult to reconcile radical reactions of these types with the salient facts concerning ketone oxidation with hydrogen peroxide and the per-acids, amongst which the formation of (5) from α-tetralone is quite typical.

Baeyer and Villiger (*Ber.*, 1899, **32**, 3625; 1900, **33**, 862) found that Caro's acid reacted with cyclic ketones to give cyclic lactones, carbon–carbon bond fission taking place with the addition of an atom of oxygen to the ring. The reaction has been found to be quite general; other per-acids, *e.g.*, perbenzoic, peracetic, and perphthalic acids, can be used similarly:



Marker (*J. Amer. Chem. Soc.*, 1940, **62**, 525, 2543) has used the reaction to convert $\gtrless C{\cdot}CO{\cdot}CH_3$ groups into $\gtrless C{\cdot}O{\cdot}CO{\cdot}CH_3$ groups, and Dilthey, Inckel, and Stephan (*J. pr. Chem.*, 1940, **154**, 219) have shown that Caro's acid converts benzophenone into phenyl benzoate. Since Aryl–CO–Alkyl is regularly converted into Aryl–O–CO–Alkyl, the mechanism of the reaction cannot involve the addition of hydroxyl radicals to the enolic form of the ketone, as suggested by Triebs (*Ber.*, 1939, **72**, 7, 1194). Again, acetone, which easily enolises in the presence of acids, is not readily oxidised by hydrogen peroxide, even in the presence of a ferrous salt, but gives quite a stable, polymeric peroxide. Moreover, Karrer and his colleagues (*Helv. Chim. Acta*,

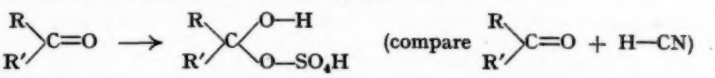
1946, **29**, 1836; 1947, **30**, 859) have shown that the per-acids will easily oxidise unsaturated diketones to acid anhydrides which are still unsaturated, *e.g.* :



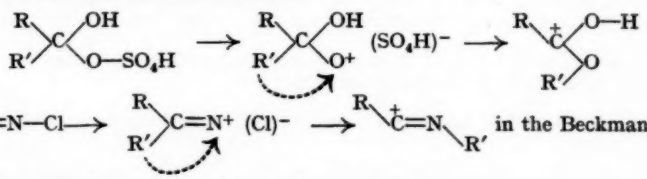
Consequently, since free hydroxyl radicals promptly attack olefinic bonds (Baxendale, Evans, and Park, *Trans. Faraday Soc.*, 1946, **42**, 155), these reactions cannot involve free hydroxyl radicals.

Now, in discussing the mode of fission of cyclic ketones, such as menthone, Baeyer and Villiger commented on the fact that the rupture of the carbon–carbon bond by Caro's acid always occurred in the same place as the rupture effected by the Beckmann rearrangement of the corresponding oximes, and suggested that both processes proceeded by a similar mechanism. This surmise gives a clear lead to a rational *ionic* mechanism for the reactions of the per-acids, which may be formulated as under :

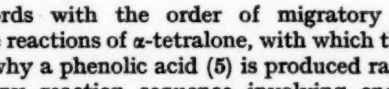
(i) The ions, H^+ and $(O{\cdot}SO_4H)^-$ of Caro's acid add to the carbonyl group of a ketone in the normal way : many ketone peroxides of this type are known.



(ii) The stable *anion* [*i.e.*, $(SO_4H)^-$ in this example] of the per-acid separates as a free entity, leaving an electron-deficient oxygen atom. This is at once stabilised by the intramolecular migration of an anionoid hydrocarbon radical.



(iii) Finally, by loss of proton, a stable lactone is formed :



This interpretation accords with the order of migratory aptitudes of the different groups, and, in the case of the reactions of α-tetralone, with which this paper is more particularly concerned, explains at once why a phenolic acid (5) is produced rather than the alternative (6), which would result from any reaction sequence involving enolisation, epoxide formation (compare Stoll and Scherrer, *Helv. Chim. Acta*, 1930, **13**, 142), or free-radical reaction, such as that involved in its autoxidation.

Experimental.

Oxidation Products of Tetralin.—The thick oil obtained by oxidation of tetralin was carefully heated at 130—150° until all the peroxide had decomposed, and then was distilled into the following fractions under a reduced pressure of nitrogen or carbon dioxide :

(i) B. p. 88°/15 mm. : this was mainly tetralin, but colour reactions (Robertson and Waters, *J.*, 1947, 496) indicated that it also contained 1 : 2-dihydronaphthalene; the liquid rapidly absorbed bromine at 0° in amount corresponding to 2% of this substance.

(ii) B. p. 130°/13 mm. : this was a pale yellow oil of peppermint odour, from which α-tetralone was separated as the oxime, m. p. 103°. A portion was refluxed with phenyl *iso*cyanate for 2 hrs., and then steam distilled. The residue was extracted with light petroleum (b. p. 60—80°) and gave the phenylurethane of α-tetralol, m. p. and mixed m. p. 122° (*ex* EtOH). Previous workers have often failed to detect α-tetralol in this fraction of liquid. Its phenylurethane is so soluble that it cannot be separated from tetralone, etc., unless all the volatile material is first steam distilled away. Separation of α-tetralol was also effected by treating the oil with finely-ground calcium chloride. This forms a solid complex with α-tetralone which can be removed by filtration, and leaves reasonably pure α-tetralol. The complex is immediately decomposed by water.

(iii) B. p. 128°/0·4 mm. : this viscous yellow product, with aldehydic reactions (Fehling's solution and Schiff's reagent), gave a 2 : 4-*dinitrophenylhydrazone*, m. p. 149° (Found : C, 56·0; H, 4·9; N, 16·0. $C_{16}H_{16}O_8N_4$ requires C, 55·8; H, 4·7; N, 16·3%), and a bisulphite compound. Analysis of the oil agreed with that for γ-*o*-*hydroxyphenylbutaldehyde* (4) (Found : C, 73·2; H, 7·1. $C_{10}H_{12}O_2$ requires C, 73·1; H, 7·3%). On exposure to air the substance was slowly oxidised to the corresponding acid (5, see below), m. p. 68°.

(iv) B. p. 128—200°/0·4 mm. : this mixture could not be separated by repeated fractional distillation, probably on account of progressive lactonisation or esterification. It was acidic, and when treated with sodium hydroxide gave a transient blue-green colour and finally became brown. Acidification of the alkaline extract gave a dark brown oil which, by extraction with carbon disulphide gave a water-soluble carboxylic acid which coloured ferric chloride bluish-green and coupled to a red dye with diazotised sulphanilic acid. It was finally obtained as a solid, m. p. 68°, and proved to be identical with γ-*o*-hydroxyphenylbutyric acid, which was prepared, for comparison by Schroeter's method, as follows : 40 G. of finely powdered potassium persulphate were mixed at 0° with 40 g. of concentrated sulphuric acid and then added slowly, with vigorous mechanical stirring, to an ice-cold solution of 15 g. of α-tetralone in 40 g. of methanol. When reaction was complete, the excess of acid was neutralised, the bulk of the methanol was removed by distillation, and the residual methyl ester of (5) was extracted with ether and purified by distillation (b. p. 180°/15 mm.). The free acid, m. p. 68°, tended to lose water on heating (Found : C, 66·2; H, 6·9; equiv., 180. Calc. for $C_{10}H_{12}O_3$: C, 66·2; H, 6·7%; equiv., 180).

(v) B. p. > 200°/0·4 mm. : this was a very viscous, dark brown, liquid from which, by alkali extraction β-*o*-carboxyphenylpropionic acid, m. p. 164°, was obtained (Found : C, 62·5; H, 5·1; equiv., 100. Calc. for $C_{10}H_{10}O_4$: C, 62·0; H, 5·1%; equiv., 97). Chromatographic analysis of the residue was attempted but did not give pure products. Polymerised hydrocarbons were evidently present.

Autoxidation of cyclo*Hexanone.*—This was exceedingly slow at 76° but proceeded at 100°. After 10 hours' aeration the liquid gave a heavy crystalline deposit of adipic acid, m. p. 151°, when cooled. The filtrate from this was washed 3 times with ice-cold potassium hydroxide solution and the extract was shaken with ether to remove dissolved *cyclo*hexanone. After careful neutralisation at 0°, the solution was extracted with ether to separate cyclohexane-1 : 2-dione (compare Riley, Morley, and Friend, *J.*, 1932, 1879). The extract, after drying, gave, besides adipic acid, an oil, b. p. <100°/20 mm., which formed an oxime that gave with nickel sulphate the flocculent red precipitate characteristic of a glyoxime.

Distillation of the direct product of oxidation gave a small fraction of a carboxylic acid, b. p. 145—150°/10 mm., which also gave positive tests for the aldehyde group (Schiff's reagent, etc.). The fraction gave a dinitrophenylhydrazone which, after recrystallisation from alcohol, had m. p. 141°, identical with that obtained by Baer (*J. Amer. Chem. Soc.*, 1942, **64**, 1416) for adipic hemi-aldehyde.

Autoxidation of α-Tetralone.—This was conducted at 100° and gave an acidic orange-red product which contained peroxides. The liquid was extracted with ice-cold sodium hydroxide solution, the extract neutralised, and the liberated ketone converted into the oxime, as described above. Addition of nickel sulphate solution to this gave a reddish-brown precipitate, which was slightly soluble in chloroform, giving a pink solution.

β-Tetralone soon deposited crystals when exposed to the air, and after purification, these proved to be β-*o*-carboxyphenylpropionic acid, m. p. and mixed m. p. 164°.

One of us (A. R.) thanks Messrs. Imperial Chemical Industries (Billingham) Ltd. for assistance whilst participating in this work.

THE DYSON PERRINS LABORATORY, OXFORD. [*Received, October 28th,* 1947.]

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318. *Studies of the Autoxidation of Tetralin. Part II. The Decomposition of Tetralin Hydroperoxide.*

By ALAN ROBERTSON and WILLIAM A. WATERS.

The decomposition of pure tetralin hydroperoxide has been examined quantitatively. In both tetralin and chlorobenzene solutions it is substantially a first-order reaction, but at high concentrations there are divergencies from the simple homolysis $C_{10}H_{11}\cdot O\cdot OH \longrightarrow C_{10}H_{11}O\cdot + \cdot OH$ which indicate that chain reactions of ·OH, etc., are to some extent involved.

Methods have been developed for the analysis of the various decomposition products, and these show that the production of ·OH and $C_{10}H_{11}O\cdot$ radicals is followed both by intramolecular changes (giving α-tetralone and water) and by true free-radical reactions (giving α-tetralol, oxygen, and other products). The Franck–Rabinowitch " cage effect " of solvent molecules is invoked to explain the formation of a constant percentage of α-tetralone. The decomposition has also been studied in the presence of cobalt naphthenate. Though then much accelerated, it still has substantially the same kinetic characteristics, and yields the same range of products.

OUR earlier studies of the autoxidation of tetralin (*Trans. Faraday Soc.*, 1946, **42**, 201) indicated that decomposition of tetralin hydroperoxide provides free radicals which act as chain-starters for the oxygen-uptake process. It therefore seemed probable that the uncatalysed thermal decomposition of tetralin hydroperoxide in solution might involve (*a*) the slow unimolecular homolysis (1) followed by (*b*) more rapid reactions of free hydroxyl and of free alkoxyl radicals.

$$C_{10}H_{11}\cdot O\cdot OH \longrightarrow C_{10}H_{11}\cdot O\cdot + \cdot OH \quad . \quad . \quad . \quad . \quad . \quad (1)$$

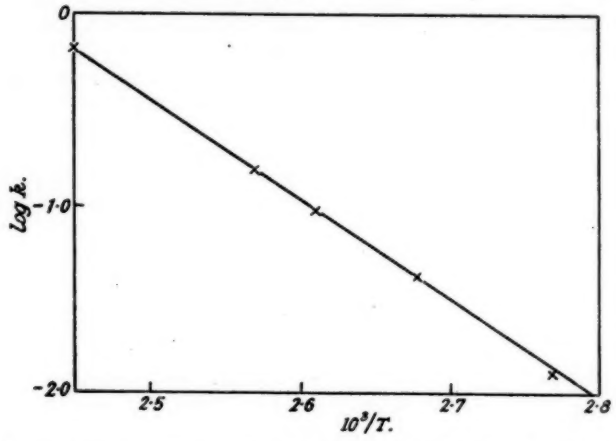
Moreover, it seemed plausible to hope that a study of the kinetics of the decomposition of tetralin hydroperoxide might provide a quantitative measure of the rate of the chain-starting reaction of the oxygen-uptake cycle. The present work, however, has revealed that the thermal decomposition of tetralin hydroperoxide is far more complex than was at first thought.

Kinetic studies of the rate of decomposition of tetralin hydroperoxide were first carried out in tetralin solution under a nitrogen atmosphere which prevented concurrent autoxidation of the solvent. The results, at first, indicated that the decomposition was a first-order reaction, for at a series of temperatures ranging from 88° to 135° excellent linear plots of log (peroxide destroyed) against reaction time were obtained. These gave the first-order reaction-velocity constants which have been plotted Fig. 1, and correspond to the Arrhenius equation

$$k = 2{\cdot}27 \times 10^{9} e^{-24{,}400/RT} \text{ g.-mol./l./sec.}$$

Heats of activation, ranging from 20 to 30 k.-cals. for the fission of the O–O link in analogous peroxides have been recorded by many other workers. Bolland (*Proc. Roy. Soc.*, 1946, *A*, **186**, 218) gives 26 k.-cals. as the activation energy for the second-order decomposition of the hydroperoxide of ethyl linoleate, though, with Gee (*Trans. Faraday Soc.*, 1946, **42**, 244), he estimates the O–O covalent bond strength as 66 k.-cals.

FIG. 1.



However, the thermal decomposition of tetralin hydroperoxide in solution is not a simple unimolecular homolysis corresponding to equation (1). Ivanov, Savinova, and Mikhailova (*Compt. rend. Acad. Sci. U.R.S.S.*, **1939**, **25**, 34) had reported that the velocity of decomposition of tetralin hydroperoxide depends upon the nature of the solvent used. This fact became more significant when a series of solvents of different chemical type were investigated, as shown by our results in Table I. This marked solvent influence indicates that the free radicals produced by the decomposition process (1) react with the solvent, and initiate chain reactions which by some means bring about a radical-catalysed decomposition of the tetralin hydroperoxide which proceeds concurrently with its uncatalysed decomposition (compare Nozaki and Bartlett's kinetic studies of the decomposition of dibenzoyl peroxide, *J. Amer. Chem. Soc.*, 1946, **68**, 1686).

TABLE I.

Solvent effects on the rate of decomposition of tetralin hydroperoxide.

Solvent.	% Hydroperoxide decomposed after 1 hour at 116°.	Solvent.	% Hydroperoxide decomposed after 1 hour at 116°.
Tetralin	10	*cyclo*Hexanone	41
Chlorobenzene.....	10	*cyclo*Hexanol	78
o-Dichlorobenzene	30	α-Chloronaphthalene.....	100

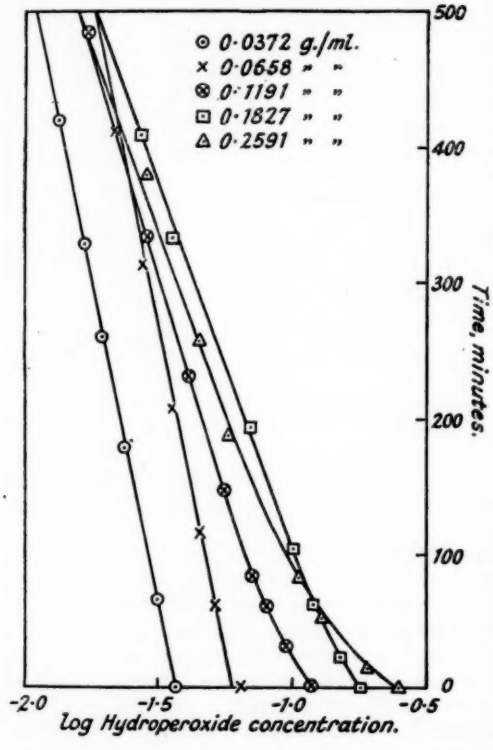
Radical-catalysed decompositions of this type should become more pronounced the greater the concentration of the dissolved hydroperoxide, since only when the solute is present in very low concentration can it safely be presumed that all the active free radicals generated by process (1) will react with, and be rendered inert by, solvent molecules. At higher concentrations chain reactions, such as (2) may occur :

$$\left.\begin{aligned} \cdot OH + R''CH\cdot O\cdot OH &\longrightarrow H_2O + R''\dot{C}\cdot O\cdot OH \\ R''\dot{C}\cdot O\cdot OH &\longrightarrow R''C{=}O + \cdot OH \end{aligned}\right\} \textit{chain} \quad \ldots \quad (2)$$

Fig. 2 shows that this concentration effect does complicate the first-order decomposition of tetralin hydroperoxide. The decomposition appears to be strictly of first order at concentrations below 0·12 g./ml. but becomes noticeably more rapid at higher concentrations.

These physicochemical studies, however, give us no more than a superficial knowledge of the reaction process. Its real complexity has been revealed by quantitative study of the decomposition products formed. The compounds described in Part I (previous paper) were all isolated from the mixture obtained by decomposing tetralin hydroperoxide in tetralin under nitrogen. α-Tetralone and α-tetralol are both major products of the decomposition, and quantitative methods for their estimation were worked out (see Experimental). Some previous workers have either failed to detect α-tetralol (*e.g.*, Medvedev and Podyapolskaya, *J. Phys. Chem. Russia*, 1939, **12**, 719) or have confidently assumed that it was not formed (*e.g.*, George and Robertson, *Proc. Roy. Soc.*, 1946, *A*, **185**, 309) and have, in consequence, been seriously misled in their interpretations of the kinetics of autoxidation. The presence of α-tetralol in this reaction has, however, been noted by Sully (*Trans. Faraday Soc.*, 1946, **42**, 261) and by the author of French Patent 37,351 of 1929. We have confirmed Sully's statement that the α-tetralone which is obtained from tetralin by the standard autoxidation procedure (*Org. Synth.*, **20**, 94) contains about 20% of tetralol, which cannot be separated by distillation. In order to obtain pure α-tetralone it was necessary to prepare its crystalline semicarbazone (Kipping and Hill, *J.*, 1899, **75**, 144) and regenerate the ketone from this.

FIG. 2.



Water is always formed in appreciable amounts. It was estimated on the small scale by the cinnamoyl chloride method (van Niewenberg, *Microchim. Acta*, 1937, **1**, 71) since the reaction mixtures were too deeply coloured for the use of the Karl Fischer reagent. Larger-scale confirmations were carried out by the Dean and Stark method. Rather unexpectedly, it was found that *oxygen* is evolved during the thermal decomposition of tetralin hydroperoxide in inert solvents such as chlorobenzene. This observation is of special significance in relation to the kinetics of autoxidation (Part III) since it indicates that the critical chain-breaking reaction may be (3). However, in the decomposition of tetralin hydroperoxide, under nitrogen, the

$$R''CH{\cdot}O{\cdot}O{\cdot} + {\cdot}OH \rightleftharpoons R''CH{\cdot}OH + O_2 \quad . \quad . \quad . \quad . \quad . \quad (3)$$

oxygen evolution is far too small to account for the whole of the production of α-tetralol along the lines of equation (3). Most of the tetralol must be derived from $C_{10}H_{11}O{\cdot}$ radicals which have oxidised (*i.e.*, dehydrogenated) other organic material.

Our analytical results, which were quite reproducible, are given in Table II. Figures obtained for the decomposition of tetralin hydroperoxide in tetralin solution (under nitrogen) were identical with those for its decomposition in chlorobenzene.

TABLE II.

The decomposition products of tetralin hydroperoxide, moles %.

Ketones (calc. as α-tetralone)	48·0
Alcohols (calc. as α-tetralol)	30·0
Acids (calc. as monobasic)	7·5
Saponifiable material	5·0
Water (Dean and Stark method)	55·5
" (cinnamoyl chloride method)	54·5
Oxygen	1·5

It has often been suggested that epoxides are possible autoxidation products of hydrocarbons. However, prolonged hydrolysis of the decomposition mixture with dilute acid did not alter appreciably the ketone/alcohol ratio, so that epoxides cannot be present in significant bulk in the finally decomposed mixture. Attempts to isolate diols as their phenyl- or naphthyl-urethanes failed completely. It may be suggested that under the conditions of the reaction any tetralin epoxide which might have been formed would tend to isomerise to β-tetralone, which would then be oxidised rapidly. It should be noted, however, that our figures for alcohols include the phenolic acids (5) and (6) of Part I, though to balance this slight error " saponifiable material " will include some tetralyl esters. The net figure of 30 moles % of α-tetralol cannot therefore be much in error.

We have been unable to substantiate Medvedev's suggestion (*Acta Physicochim. U.R.S.S.*, 1938, **9**, 395) that tetralin hydroperoxide and α-tetralone combine to form a stable hydroxyperoxide. In fact, the decomposition of tetralin hydroperoxide is rapid in ketonic solvents, and a concentrated solution (20%) of tetralin hydroperoxide gave a slightly higher yield (49·5%) of α-tetralone than did one half of this strength (see Table II).

Evidence for the Presence of Free Radicals during the Decomposition of Tetralin Hydroperoxide.—(1) Table III shows that tetralin hydroperoxide and dibenzoyl peroxide are comparable as catalysts for initiating the chain polymerisation of olefins. This is a reaction which involves the presence of active free radicals.

TABLE III.

Tetralin hydroperoxide as a catalyst for polymerisation processes.

Polymerisation.	Peroxide, %.		Induction period, mins.	Appearance.
Styrene: heated at 115° in sealed tubes; air present	Tetralin hydroperoxide	1	150	Solidified on cooling
		5	120	
	Benzoyl peroxide	1	95	,,
		5	55	
	None		180	Still mobile
Methyl methacrylate: heated at 50°; air atmosphere	Tetralin hydroperoxide	1	145	Viscous liquid
		5	65	
	Benzoyl peroxide	1	30	,, ,,
		5	25	
	None		180	Mobile
Vinyl cyanide: heated at 55°; air present	Tetralin hydroperoxide	1	1	Polymer deposited
		5	5	
	Benzoyl peroxide	1	105	,, ,,
		5	19	
	None		120	Still clear

(2) It has been suggested by one of us (Waters, *Trans. Faraday Soc.*, 1946, **42**, 184; *Nature*, 1946, **158**, 380) that the splitting of α-glycols to aldehydes and ketones by lead tetra-acetate, or by Fenton's reagent, is due to their dehydrogenation by free $\cdot O \cdot CO \cdot CH_3$ or ·OH radicals. The thermal decomposition of tetralin hydroperoxide at 135° in various glycols was therefore examined. Both ethylene glycol and glycerol yielded formaldehyde, 2 : 3-butylene glycol yielded acetaldehyde, and pinacol yielded acetone. Under the same conditions, *cyclo*hexanol, previously freed from ketone by distilling it from 2 : 4-dinitrophenylhydrazine, gave some *cyclo*hexanone. Presumably some of the tetralone originates in this way from tetralol.

(3) Attempts to establish the presence of the free hydroxyl radical by its substitution in benzene, chlorobenzene, chloronaphthalene, nitrobenzene, *p*-nitrotoluene, and *m*-dinitrobenzene were also made, but the corresponding phenolic compounds could not be isolated, though Milas (*J. Amer. Chem. Soc.*, 1937, **59**, 2342) has reported that aromatic compounds can be hydroxylated with *tert.*-butyl hydroperoxide. It may be concluded, therefore, that the phenolic by-products of the decomposition of tetralin hydroperoxide are not formed by free-radical hydroxylation, but rather by the molecular rearrangement process suggested in Part I.

The Decomposition of Tetralin Hydroperoxide in the Presence of Cobalt Naphthenate.—Cobalt naphthenate, which is one of the most active of the metal-salt catalysts of autoxidation, is also a potent catalyst for the decomposition of tetralin hydroperoxide (compare Ivanov, Savinova, and Mikhailova, *loc. cit.*; Cook, *J.*, 1938, 1774). Fig. 3 shows that, in tetralin solution at 20°, the catalysed decomposition is still of first order with respect to tetralin hydroperoxide until about 90% of the material has been destroyed, but thereafter the reaction rate shows signs of decreasing. This may be due to a slow decomposition of the catalyst, for solid is eventually deposited from

these solutions. Fig. 4 shows that, provided only small amounts of catalyst be added, the rate of decomposition of tetralin hydroperoxide is also of first order with respect to cobalt naphthenate (compare George, Rideal, and Robertson, *Proc. Roy. Soc.*, 1946, *A*, **185**, 288).

FIG. 3.

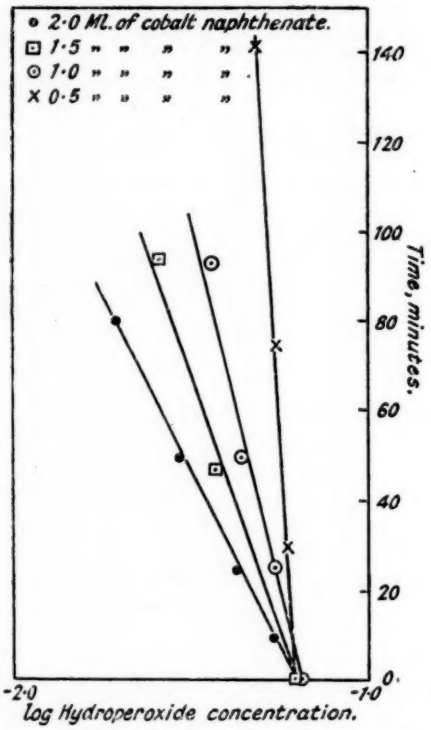
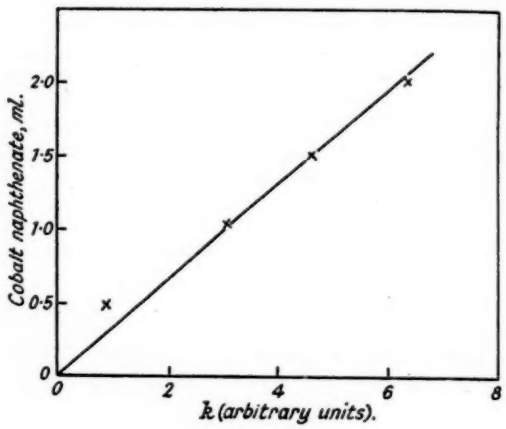


FIG. 4.



Immediately tetralin hydroperoxide is added to a reddish-brown solution of cobalt naphthenate in any organic solvent the colour changes to a pale yellow, and decomposition sets in. This colour change is probably an indication of the oxidation of the cobaltous ion to a higher valency state (*e.g.*, Co^{+++}).

TABLE IV.

The decomposition of tetralin hydroperoxide in the presence of cobalt naphthenate at 76°.

Product.	Yield, moles %.	Product.	Yield, moles %.
Ketones (as α-tetralone)	48·0	Saponifiable material	1·0
Alcohols (as α-tetralol)	33·0	Oxygen evolved.....	16·1
Acids (as monobasic).....	2·0		

Table IV, which gives the results of a quantitative analysis of the products of the catalysed decomposition in the presence of 4% of cobalt naphthenate, shows that the catalysed decomposition, like the uncatalysed decomposition (Table II), is still a complex process. An increase in the quantity of catalyst had no significant effect upon the ketone/alcohol ratio.

A comparison of Tables II and IV shows that the relative yields of the two main decomposition products, *viz.*, α-tetralone and α-tetralol, are very little altered by the presence of the catalyst. Much more oxygen is evolved in the catalysed decomposition, however, and correspondingly the quantity of complex oxidation products (acid and saponifiable material) becomes less. In fact, the quantity of oxygen evolved in the catalysed decomposition becomes nearly equivalent to the amount of α-tetralol formed, suggesting that the reaction $2C_{10}H_{11}\cdot O\cdot OH = 2C_{10}H_{11}\cdot OH + O_2$ has now become important.

Since cobalt naphthenate strongly catalyses the autoxidation of tetralin, it may be inferred that peroxide radicals, $C_{10}H_{11}\cdot O\cdot O\cdot$, are abundant in these decomposing mixtures. Consequently,

the following reaction sequence, which accords with the chain-ending process to be discussed in Part III (following paper), may be suggested in order to account for this large oxygen evolution.

$$C_{10}H_{11}\cdot O\cdot OH \longrightarrow C_{10}H_{11}\cdot O\cdot + \cdot OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

$$C_{10}H_{11}\cdot O\cdot OH + \cdot OH \text{ (or } \cdot OR) \longrightarrow C_{10}H_{11}\cdot O\cdot O\cdot + H_2O \text{ (or HOR)}$$

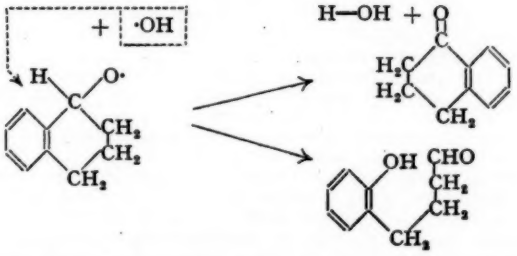
$$C_{10}H_{11}\cdot O\cdot O\cdot + \cdot OH \longrightarrow C_{10}H_{11}\cdot OH + O_2 \text{ (chain-ending)}$$

In these circumstances, the hydroperoxide decomposes so rapidly that the radicals $C_{10}H_{11}O\cdot$ and $\cdot OH$ are present in high concentration. The reaction chains in which they are concerned then become very short, on account of the high probability of mutual encounter of two radicals, and in the main lead to the conversion of tetralin hydroperoxide into α-tetralol and oxygen. In the absence of a catalyst the hydroperoxide decomposes so slowly that the radicals, formed only in very low concentration, have a greater chance of dispersing, and of reacting with other solute molecules, *e.g.*, of oxidising tetralone to acid substances.

The foregoing suggestion depends upon the proposition that free radicals are produced during the metal-salt-catalysed decomposition of tetralin hydroperoxide. This has been supported by showing that glycol fission (of ethylene glycol, glycerol, 2 : 3-butylene glycol, and pinacol) could be effected, even at moderate temperatures, in the presence of the cobalt naphthenate catalyst.

Discussion.—Our kinetic work has indicated that both in absence and in presence of a metal-salt catalyst the thermal decomposition of tetralin hydroperoxide is substantially a first-order reaction, giving a complicated mixture of products such as one would expect from a homolytic reaction in which free neutral radicals had been produced. The natures of the subsidiary oxidation products and the evidence of glycol fission both suggest that these free radicals are hydroxyl and alkoxyl ($C_{10}H_{11}\cdot O\cdot$). Nevertheless, in all circumstances about half the tetralin hydroperoxide seems to decompose according to the simple equation $C_{10}H_{11}\cdot O\cdot OH = C_{10}H_{10}O + H_2O$, whilst a slight amount seems to undergo a molecular rearrangement to an isomer, γ-*o*-hydroxyphenylbutaldehyde [(4) of Part I]. Although these products may in part be produced by free-radical reactions (*e.g.*, by the oxidation of α-tetralol), it is much more likely that they both result from intramolecular decompositions.

In order to explain why the quantum efficiencies of photolyses in solution were usually very low indeed, Franck and Rabinowitch (*Trans. Faraday Soc.*, 1934, **30**, 120) pointed out that whenever a molecule decomposed into two radicals, within a liquid, then the dissociated fragments would tend to be driven together again by the collisions of the surrounding solvent molecules, and only in the exceptional circumstances of their possessing high translational energy would they be able to drift far apart before recombining. Now this " cage effect " of solvent molecules will explain very well the high, and constant, yield of α-tetralone in the thermal decomposition of tetralin hydroperoxide, for, following the simple homolysis (1) (p. 1578), there may occur (*a*) direct recombination of radicals, as postulated by Franck and Rabinowitch, or (*b*) the reactions illustrated below, in which the radicals have had sufficient independent existence to change their relative alignments by translational motion, but not the opportunity to become really " free ".



The formation of γ-*o*-hydroxyphenylbutaldehyde has its direct analogy in the decomposition of diphenylmethane hydroperoxide to benzaldehyde and phenol, noted by Hock and Lang (*Ber.*, 1944, **77**, 257), and in the decomposition of triphenylmethane hydroperoxide to benzophenone and phenol (Wieland and Maier, *Ber.*, 1931, **64**, 1205).

The direct recombination (*a*) would merely have the effect of reducing the observed velocity of decomposition of tetralin hydroperoxide below that which one might calculate on the supposition that bond fission occurs whenever the molecule gets enough activation energy. This,

of course, will be common to all decompositions in solution. It accords with the observed low value ($2{\cdot}27 \times 10^9$) of the reaction constant (p. 1579).

The cage effect (*b*), though structural in the sense that different molecules would undergo intramolecular processes of different types, again depends in magnitude upon the probability of separation of a pair of freshly-formed radicals, but should be independent of the molar concentration of the decomposing substance, though possibly dependent upon the temperature and upon the nature of the solvent.

Consequently, the rate of production of a free radical of any one species can be written as

$$+ \mathrm{d[OH]}/\mathrm{d}t = \rho k[\text{Hydroperoxide}]$$

where ρ represents the fraction of freshly-formed radicals which become " free " enough to act as chemical reagents, or as chain-starting catalysts, and k is the measured unimolecular decomposition-velocity constant. Part III seems to indicate that only one of the free radicals produced by the thermal decomposition of tetralin hydroperoxide is catalytically active. In this following paper the factor k_c is used to represent the product ρk.

The reaction mechanism for the catalysed decomposition has already been considered by us (*Trans. Faraday Soc., loc. cit.*), and our previous conclusion, *viz.*, that it is a chain reaction in which the metal cation undergoes reversible one-electron oxidation–reduction (*e.g.*, $Co^{++} \rightleftharpoons Co^{+++} + e$) does not need substantial modification, though it now becomes necessary to stress the fact that the reaction chains involving the radicals ·OH and $C_{10}H_{11}O\cdot$ must all be short. It is noteworthy, however, that this consideration of the catalysed reaction led to the conclusion that the catalytically active radical was free hydroxyl, ·OH, and not $C_{10}H_{11}O\cdot$; though this view was based upon the report that tetralone rather than tetralol was formed in quantity. Though the true nature of the active catalyst radical in autoxidation is still in doubt between these two alternatives we may point out that, as a chain-breaking reaction, the process $C_{10}H_{11}\cdot O\cdot O\cdot + \cdot O\cdot C_{10}H_{11} \longrightarrow O_2 + C_{10}H_{11}\cdot O\cdot C_{10}H_{11}$ does not seem to occur to any appreciable extent, because we have been unable to isolate ditetralyl ether from any of our reactions.

Experimental.

Preparation of Pure Tetralin Hydroperoxide.—Tetralin containing 0·01% of cobalt naphthenate as catalyst was charged into a flask equipped with a reflux condenser, with ground glass joints, and a glass frit gas-distributor spoon. (Rubber connections must be avoided, as rubber is very quickly destroyed by tetralin.) The tetralin was then air-blown for 25—30 hours. The resulting solution (*ca.* 25% peroxide) was then distilled in an all-glass apparatus under nitrogen at 1 mm., a water-bath being used which was not heated to above 60°, and about 80% of the tetralin was removed at about 52°. The concentrate was stored overnight at − 10°, and the resulting crystalline product was collected on a sintered-glass filter, pressed dry, and crystallised at least three times from light petroleum (b. p. 40—60°). The pure product melted at 56°; yield 17—20%.

Estimation of Tetralin Hydroperoxide (in kinetic studies, etc.).—In a conical flask, fitted by a ground glass joint to a glass bubble-trap containing potassium iodide solution, were placed 20 ml. of glacial acetic acid, 1·5 g. of sodium hydrogen carbonate, and just sufficient water to cause carbon dioxide evolution (a few drops only). The sample for analysis was added from a syringe pipette, which was rinsed out with glacial acetic acid, the rinsings being added to the flask also. A slight excess of 50% potassium iodide solution was then added, the bubble-trap placed in position, and the flask heated at 80° for 15 mins. After this had been kept in the dark for 1 hour, the combined contents of both the flask and the trap were diluted with water, and titrated with thiosulphate, starch being used as indicator. Very viscous samples were syringed into a tared, dry flask, which had been purged with nitrogen to prevent oxidation, stoppered, and weighed. The estimation was then completed by adding acetic acid and potassium iodide and proceeding as above.

Under these conditions our purest tetralin hydroperoxide regularly gave 97% of the calculated quantity of iodine. Only 85% reaction occurred if mixtures were merely left in stoppered flasks for 24 hours. The prompt fitting of the bubble-trap was essential to secure reproducibility and accuracy.

Kinetic Measurements.—Kinetic measurements of the decomposition of tetralin hydroperoxide in solution were carried out in 50-ml. tubes, fitted with a glass stopper, and provided with gas inlet and exit tubes. These reaction tubes were heated to constant temperatures by immersion in the vapours of suitable refluxing liquids (trichloroethylene, water, toluene, *n*-butanol, and ethylbenzene). In each experiment about 1 g. of pure tetralin hydroperoxide was weighed accurately into a reaction tube and dissolved in 15 ml. of tetralin or other solvent, air was displaced by nitrogen, the stopper inserted, and the tube then placed in its vapour-bath. A very slow stream of nitrogen was maintained throughout. Periodically, samples were withdrawn by means of a syringe pipette. Six or more samples were taken in each experiment at times which covered the range 10—90% decomposition. Good logarithmic plots, resembling those in Fig. 2, could always be constructed. Duplicate runs were concordant.

Quantitative Methods for the Analysis of Reaction Products.—(1) *α-Tetralone.* (*a*) Gravimetric method. The weighed sample of oil was dissolved in 30 ml. of 50% ethanol and treated with an excess of a filtered solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid, with vigorous shaking. After 1 hour the solution was filtered through a sintered-glass crucible, and washed well with the acid and finally with distilled water. It was weighed after being dried at 100° : 1 g. of dinitrophenylhydrazone corresponds to 0·4478 g. of α-tetralone. This method was practicable only for samples of high purity. In presence

of tetralin the dinitrophenylhydrazone formed a red oil which could not be filtered. Tetralin hydroperoxide oxidised the reagent.

(*b*) Volumetric method. This was much more widely applicable, but again peroxide had to be absent. (Omission of this precaution vitiates nearly all the analytical results of Larsen, Thorpe, and Armfield, *Ind. Eng. Chem.*, 1942, **34**, 282.) 20 Ml. of a 20% solution of pure hydroxylamine hydrochloride in 80% methanol, and 20 ml. of methanol were added to each of two conical flasks. The solutions were adjusted to neutrality to dimethyl-yellow indicator (used as a 0·1% methanolic solution), N/10-methanolic sodium hydroxide being used. The sample to be analysed was added to one flask, and both flasks were then heated, under reflux condensers, for 2 hours on a water-bath. After cooling, both samples were titrated to neutrality with N/10-methanolic sodium hydroxide, the same indicator being used. 1 Ml. of this alkali is equivalent to 0·0146 g. of α-tetralone.

With pure α-tetralone (*ex* its semicarbazone) this method gave 98·5% of the theoretical value. Bromophenol-blue (pH range 3·0—3·6) could be used in place of dimethyl-yellow (pH range 2·8—4·6). When deeply-coloured oxidation products were being examined this colorimetric end-point could not be used, and the following trustworthy modification was adopted. After being refluxed, the blank solution was transferred to a beaker, a glass electrode inserted, the system connected to a calomel half-cell, and the pH determined by means of a Cambridge pH meter. This process was then repeated with the sample solution, which was titrated with the methanolic alkali until the pH was identical with that of the blank (approx. 3·5).

(2) *α-Tetralol.* The sample, from a specimen freed from water by azeotropic distillation, was refluxed on a water-bath with a suitable quantity of acetic anhydride in anhydrous pyridine, and a blank mixture was submitted to a similar treatment. After 2 hours' refluxing, both samples were well diluted with distilled water and titrated with N/5-alcoholic sodium hydroxide with phenolphthalein as indicator. Allowance was made for the free acid in the sample: 1 ml. of N/5-sodium hydroxide corresponds to 0·0296 g. of α-tetralol. By this method pure α-tetralol gave 97% of the theoretical titre, and so did equimolar mixtures of tetralol and tetralone.

(3) *Water.* The Dean and Stark method was used for the decomposition of 16—20-g. samples of tetralin hydroperoxide dissolved in 75 ml. of dried chlorobenzene, a receiving vessel calibrated to 0·05 ml. being used.

For the cinnamoyl chloride method, 1 g. of tetralin hydroperoxide was dissolved in 2·5 ml. of sodium-dried tetralin, and heated, in a slow stream of phosphoric oxide-dried nitrogen, in a small flask immersed in oil at 140°. The gases were passed through a bubbler containing cinnamoyl chloride maintained at 40°, and thence into distilled water, where the hydrochloric acid, equivalent to the water produced in the decomposition, was collected and finally titrated with N/10-sodium hydroxide.

(4) *Oxygen.* Decompositions were carried out in boiling chlorobenzene solution. a slow stream of purified nitrogen being used. The gases were collected and measured, and their oxygen content was determined by absorption in pyrogallol.

Catalysed Decompositions.—The cobalt naphthenate catalyst was a typical technical product, taken from a stock of material normally used as an autoxidation catalyst. A stock 1% solution in tetralin was made, by prolonged shaking in a closed bottle, and portions were filtered before use. The solution gradually deteriorated over a period of months, and reproducible results could not be obtained from samples which had been exposed to the air.

Glycol Fision Experiments.—The decomposition of solutions of tetralin hydroperoxide in a number of glycols was carried out both by thermal treatment at 135° and by the addition of cobalt naphthenate catalyst. Vapours from the solution of the decomposing hydroperoxide were blown by a nitrogen stream through an air condenser and thence to bubblers containing a suitable test reagent (dimedone or 2 : 4-dinitrophenylhydrazine). Derivatives of any aldehydes or ketones present in the decomposition products were filtered off, recrystallised, and compared with samples of authentic specimens.

One of us (A. R.) thanks Messrs. Imperial Chemical Industries (Billingham) Ltd. for assistance whilst participating in this work.

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319. *Studies of the Autoxidation of Tetralin. Part III. An Interpretation of the Kinetics of Autoxidation.*

By ALAN ROBERTSON and WILLIAM A. WATERS.

The reactions involved in the decomposition of tetralin hydroperoxide can be used to elucidate the natures of the critical chain-starting and chain-stopping reactions of the "uncatalysed" autoxidation of tetralin. Free hydroxyl radicals are probably involved both as chain-starting and as chain-stopping agents.

THE autoxidation of tetralin, like that of many other hydrocarbons, involves two consecutive, but inter-related phases, *viz.*, (I), the formation of a hydroperoxide, and (II) the breakdown of this hydroperoxide in the presence of both hydrocarbon and oxygen, to a series of other products. Now that the salient features of phase (II) of this process have been established experimentally (see Part II, preceding paper) it is possible to review their connections with phase (I) of the autoxidation process, and to interpret the course of the oxygen uptake process from the basis of a much wider range of experimental data than has been available hitherto.

The main features of the oxygen uptake process (I) have been described previously (Robertson and Waters, *Trans. Faraday Soc.*, 1946, **42**, 201; George and Robertson, *Proc. Roy. Soc.*, 1946, *A*, **185**, 309; Medvedev, *Acta Physicochim., U.R.S.S.*, 1938, **9**, 395; Larsen, Thorpe, and Armfield, *Ind. Eng. Chem.*, 1942, **34**, 182). They consist of (*A*) a period of slow, but accelerating reaction in which tetralin hydroperoxide accumulates; (*B*) a period of reaction at a constant rate in which more peroxide is formed than is decomposed; and (*C*) a final period of decreasing oxygen uptake, when a very complex reaction mixture has been formed.

During the initial, autocatalytic, period (*A*), tetralin hydroperoxide, as it is formed, catalyses the reaction. This effect is the more noticeable at low temperatures. In these circumstances Medvedev found that the rate of oxygen uptake (his " partial rate " w_2) was a function of the peroxide content of the liquid, whilst George and Robertson (*loc. cit.*, p. 316) found that it was a function of the oxygen pressure. Our measurements, made at 76°, indicate that (i) initially over 99% of the oxygen uptake corresponds to hydroperoxide formation, and (ii) whilst the reaction is still accelerating, the rate of oxygen uptake is proportional to the *square root* of the peroxide content of the liquid (see Figure). In contrast to this observation with tetralin, Bolland (*Proc. Roy. Soc.*, 1946, *A*, **186**, 218) and Bolland and Gee (*Trans. Faraday Soc.*, 1946, **42**, 236) report that for the initial stage of the autoxidation of ethyl linoleate at 35—75° the rate of oxygen uptake is proportional to the first power of the peroxide content of the liquid, but is also a function of the oxygen pressure.

Kinetics of the initial stages of autoxidation of tetralin.

Most of the studies of the autoxidation of tetralin have, however, been concerned with period (*B*), during which the oxygen is absorbed at a steady rate. In these circumstances, George and Robertson, who used very small quantities of hydrocarbon, found (i) that under 90% of the oxygen uptake corresponded to additional peroxide formation, and (ii) that the rate of oxygen uptake was proportional to the *square of the hydrocarbon concentration*, and substantially independent both of the oxygen pressure and of the peroxide content of the liquid. Their equation:

$$\text{Rate of } O_2 \text{ uptake} = k[RH]^2$$

corresponds to Medvedev's " primary process " with

$$\text{Rate } w_1 = a[RH]^2 \times f \text{ (tetralone present)}$$

Period (*B*) is also the principal reaction studied by Larsen Thorpe, and Armfield, who followed the reaction through to period (*C*), when, as pointed out by Medvedev, decomposition products of peroxides retard the reaction (compare Part I, this vol., p. 1574).

It is necessary to fit the whole of this reaction sequence into a comprehensive scheme, which must (*a*) account for the main products which are formed during both the synthesis and the breakdown of tetralin hydroperoxide, (*b*) require the postulation only of reactions involving substances (molecules, radicals, etc.) which are known from experimental evidence to be present in the system, and (*c*) take due account of the concentration and relative reactivity of each of the reacting entities throughout the successive stages of the whole autoxidation process. Thus it is essential to postulate the participation only of free radicals of types known to be present in the reacting system, and to take account only of their established chemical reactions. For instance, hypothetical reactions, such as $R{-}O{-}O\cdot + O_2 \longrightarrow R{-}O\cdot + O_3$ (George, *Trans. Faraday Soc.*, 1946, **42**, 217) must be excluded, since (i) there is no experimental evidence to support the view that free peroxide radicals can react with molecular oxygen, and (ii) ozone has not been detected as a reaction product: it is in fact an initiator of autoxidation (Briner, Demolis, and Paillard, *Helv. Chim. Acta*, 1932, **15**, 201) and not an end-product.

The Main Chain Reaction.—Chemical evidence, already set forth in detail (see Robertson and Waters, *loc. cit.*), indicates that the main oxygen uptake cycle is the *long chain* process:

(1) $R''CH\cdot + O_2 \xrightarrow{k_1} R''CH{-}O{-}O\cdot$ (*or* $R_1 + O_2 \longrightarrow R_2$)

(2) $R''CH{-}O{-}O\cdot + R''CH_2 \xrightarrow{k_2} R''CH{-}O{-}O{-}H + R''CH\cdot$ (*or* $R_2 + RH \longrightarrow P + R_1$)

since free hydrocarbon radicals (*e.g.*, phenyl) and free peroxide radicals (*e.g.*, $CPh_3{\cdot}O{\cdot}O{\cdot}$) are both *immediate* catalysts for the autoxidation of tetralin and analogous substances. In accordance with this one may write :

$$-\,d[O_2]/dt = k_1[R_1][O_2] = +\,d[P]/dt\ (\text{approx.}) = k_2[R_2][RH] \quad . \quad . \quad . \quad \textbf{(A)}$$

and must therefrom choose chain-starting and chain-stopping processes which lead to overall kinetic equations that satisfy the experimental observations.

The Chain-starting Process.—The autocatalytic nature of the primary stage of autoxidation, and the fact that tetralin hydroperoxide is an *immediate* catalyst, indicate that, in the absence of more active primary catalysts, such as dibenzoyl peroxide, tetralin hydroperoxide must be concerned in this chain-starting process in the " uncatalysed " reaction. Now the work detailed in Part II has shown that tetralin hydroperoxide generates some free radicals, probably both ·OH and $C_{10}H_{11}{\cdot}O{\cdot}$ (R·O·), in the course of its thermal decomposition in solution. Some of these active radicals undoubtedly dehydrogenate surrounding molecules, giving water, α-tetralol, and possibly other compounds as their eventual stabilised products. The dehydrogenation of tetralin by these radicals is shown by the invariable production of some 1 : 2-dihydronaphthalene during the autoxidation (see Part I). Moreover, the facts that triphenylmethyl peroxide and *tert.*-butyl hydroperoxide are both immediate catalysts for tetralin autoxidation, together with recent studies of the properties of the free hydroxyl radical (compare Merz and Waters, Faraday Society Discussion, **1947**, **2**, in the press), show that, in general R–O· radicals can attack most C–H bonds.

Neglecting for the moment any difference in reactivity between R″CH–O· and ·OH radicals, one may therefore conclude that the process

$$\textbf{(3)}\quad R_1{-}H + {\cdot}OH({\cdot}OR) \xrightarrow{k_3} R_1{\cdot} + H_2O\ (HOR)$$

does occur under the reaction conditions appertaining to autoxidation; (3) is slightly exothermic (*ca.* 6 kcals.), and its activation energy must of course be primarily dependent on the bond strength of the C–H group attacked, so that the value of 26 kcals. reported both by Medvedev and by George and Robertson for the chain-starting process in the autoxidation of tetralin does not seem to be unduly high for it.

Stages (*A*) and (*B*) of the autoxidation of tetralin, when the concentration of R–H is high and the probability of encounter of two hydroperoxide molecules, or of radicals derived therefrom, is small, are particularly the circumstances in which (3) would be a more frequently occurring reaction than any other activation process which could conceivably operate as a chain-starter to the main oxygen uptake cycle. Chemical evidence supports the choice of (3) as the chain-starting process, since small amounts of water are always formed, even in the initial stages of the autoxidations of nearly all substances.

If one postulates (3) then it follows that

$$\text{Chain-starting rate} = k_3[OH][RH] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad \textbf{(B)}$$

which accords with George and Robertson's findings that in the " uncatalysed " reaction the chain-starting process is of first order with respect to the hydrocarbon, and of zero order with respect to oxygen.

Chain-stopping Processes.—The choice of chain-stopping reactions is much more open, for there are several cogent possibilities; thus :

(i, *a*) *Dimerisation of* R_1. This should give ditetralyl, which has *not* been found as an autoxidation product, though its analogue is formed during the autoxidation of ethylbenzene (Sully, *Trans. Faraday Soc.*, **1946**, **42**, 260).

(i, *b*) *Disproportionation of* R_1. This would be kinetically indistinguishable from the above and does occur to a small extent (less than 2% of the whole reaction) since 1 : 2-dihydronaphthalene is an invariable autoxidation product of tetralin.

(ii) *Dimerisation of* R_2, *i.e.*, $2\ R{-}O{-}O{\cdot} \longrightarrow R{-}O{-}O{-}O{-}O{-}R$. This is *most* improbable, though combination with loss of oxygen is somewhat more likely. Even this, however, does not correspond with the mode of formation of oxygen from hydrogen peroxide (which can be regarded as the prototype compound for peroxide reactions). The objections under (iii) are also cogent.

(iii) *Union of* R_1 *and* R_2, *i.e.*, $R''CH{\cdot} + R''CH{-}O{-}O{\cdot} \longrightarrow R''CH{-}O{-}O{-}CHR''$. This is possible, but could occur extensively only if R_1 and R_2 were present in roughly equivalent concentration, which is seldom the case. The reaction, however, would not lead to the formation

of a stable product, but to a fresh autoxidation catalyst (compare triphenylmethyl peroxide), and thus should be ruled out as a ***main*** chain-stopping process.

Of these three reactions therefore only (i) needs serious consideration. It is not necessary, however, to confine the choice of chain-stopping reactions to processes involving only the active radicals of the main oxygen uptake cycle (1—2). Serious consideration must be given to reactions involving radicals derived from the tetralin hydroperoxide. Thus, taking the hydroxyl radical, we have :

(iv) *Direct formation of α-tetralol,* $R''CH\cdot + \cdot OH \longrightarrow R''CH{-}OH$ (or $R_1\cdot + \cdot OH \longrightarrow R_1{-}OH$).

(v) *Formation of α-tetralol by elimination of oxygen from hydroxyl and peroxide radicals,* as in the " catalase " decomposition of hydrogen peroxide :

$$R''CH{-}O{-}O\cdot + \cdot OH \longrightarrow R''CH{-}OH + O_2 \text{ (or } R_2 + \cdot OH \longrightarrow R_1{-}OH + O_2).$$

Similar equations can, of course, be derived for reactions involving alkoxy-radicals, $R{-}O\cdot$, but there is very little chemical evidence for them, since ethers, $R_1{-}O{-}R$, do not appear to be significant products of autoxidation of hydrocarbons. Much experimental evidence now indicates that alkoxy-radicals are often unstable, and so are destroyed in other ways (compare Walsh. *Trans. Faraday Soc.*, 1946, **42**, 269).

In special circumstances account should also be taken of surface reactions, or reactions with inhibitors (including molecular reaction products, see Part I, *loc. cit.*). These may for simplicity be represented as

(vi) $R_1 + I \longrightarrow$ inert products, and
(vii) $R_2 + I \longrightarrow$ inert products

since they will be essentially of first order with respect to the radical which is removed from the system.

The following consideration of these reactions indicates (*a*) that (i) may be significant during the initial autocatalytic stage (*A*) of the autoxidation, (*b*) that reaction (v) replaces (i) as the main chain-stopping process when the hydroperoxide content of liquid has risen to a sufficient extent, and (*c*) that (vii) can account for the effects of many inhibitors. In these respects our conclusions differ from those of George and Robertson (*loc. cit.*), who based their kinetic analysis of the " thermal " autoxidation of tetralin only upon the steady second stage (*B*) of the whole process and, by showing that the chain-stopping reactions (i)—(iii) were not then concerned, attempted to discredit the whole free-radical chain mechanism for the autoxidation of tetralin in the absence of added catalysts. George and Robertson proposed instead a " thermal-chain " mechanism; this we would now suggest is basically equivalent to our view, *viz.*, that the promotion of the autoxidation of tetralin is essentially due to the thermal decomposition of minute quantities of tetralin hydroperoxide. The kinetic analysis of the chemical equations which we have already given leads to the following results.

Taking, first of all, the autocatalytic stage (*A*) of autoxidation, if, in addition to the oxygen-uptake cycle of reactions (1) and (2) (p. 1586), we write

$$\text{Chain starting} = (3) = R_1{-}H + \cdot OH \xrightarrow{k_s} R_1 + H_2O$$

(4) Chain stopping = (i) = Disproportionation of R_1,

$$i.e.,\ 2R_1 \xrightarrow{k_{tA}} \text{inert products}$$

then we have the relationship

$$k_s[RH][OH] = k_{tA}[R_1]^2$$

whence

$$\frac{-d[O_2]}{dt} = k_1[R_1][O_2] = k_1[O_2]\left(\frac{k_s}{k_{tA}}[RH][OH]\right)^{\frac{1}{2}}$$

Now, when the peroxide content of our liquid is very small, and there are no oxidisable impurities in it, then the rate at which *free* hydroxyl radicals are produced depends upon the substantially first-order decomposition of tetralin hydroperoxide (see Part II), so that we may write $+ d[OH] = k_c[P]$, from which we see that so long as *all* the " free " hydroxyl radicals that emerge from the decomposition of individual tetralin hydroperoxide molecules are destroyed by reaction with the surrounding (tetralin) solvent

$$\frac{-d[O_2]}{dt} = k[P]^{\frac{1}{2}}[O_2]\left(\frac{k_s \cdot k_c}{k_{tA}}[RH]\right)^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (C)$$

The complexity of the decomposition of tetralin hydroperoxide is such that the factor k_c may possibly be a function of [RH] and of any diluent liquid, for it may be difficult to discover one which is really " inert " to hydroxyl, but this consideration does not affect our argument. Equation (**C**) should also hold for the metal-ion catalysed reaction in its initial stages, provided that we substitute k'[P][M] for k_c[P], where [M] represents the agent which promotes the homolysis of the tetralin hydroperoxide.

Equation (**C**) agrees both with our own experimental demonstration that the initial rate of oxygen uptake varies with the square root of the peroxide content of the liquid, and with the fact that at first the rate of autoxidation is dependent upon the oxygen pressure.

Passing now to the second stage (*B*) of the autoxidation, we must remember that, as the peroxide content of the liquid increases, so too will increase the rate of production of " free " hydroxyl radicals. However, though reaction (3) thereby increases, the rate of the chain-stopping reaction (4) will not change, and soon some free hydroxyl radicals will become available for taking part in reactions such as (iv) and (v). Now, both reactions (iv) and (v) satisfy the conditions that the same catalyst is involved in both the chain-starting and the chain-stopping processes. As George, Robertson, and Rideal have pointed out (*Proc. Roy. Soc.*, 1946, *A*, **185**, 288; *Nature*, 1942, **149**, 601), this is an essential feature of all autoxidations which can proceed at a steady rate. Hence either (iv) or (v) may accord with the observed kinetics of stage (*B*) of tetralin autoxidation.

Taking (3) as the chain-starting reaction and (iv) as the chain-stopping reaction, we would find

$$\frac{-\mathrm{d}[\mathrm{O_2}]}{\mathrm{d}t} = +\mathrm{d}[\mathrm{P}]/\mathrm{d}t = k_1\frac{k_3}{k_t}[\mathrm{RH}][\mathrm{O_2}] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (\mathbf{D})$$

whilst, taking (v) as the chain-stopping reaction, we would find

$$\frac{-\mathrm{d}[\mathrm{O_2}]}{\mathrm{d}t} = +\mathrm{d}[\mathrm{P}]/\mathrm{d}t = k_3\frac{k_3}{k_{tB}}[\mathrm{RH}]^2. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (\mathbf{E})$$

Chain-stopping reaction (v), *i.e.*,

(5) $\mathrm{R''CH{-}O{-}O\cdot} + \cdot\mathrm{OH} \xrightarrow{k_{tB}} \mathrm{R''CH{-}O{-}H} + \mathrm{O_2}$

thus accords with the kinetics of the steady stage (stage *B*) in the thermal autoxidation of tetralin, as observed by George and Robertson (*Proc. Roy. Soc., loc. cit.*), whilst chain-stopping by reaction (iv) corresponds to the kinetics of the metal-salt catalysed reaction at low oxygen pressures.

Reaction (5) (= v) accords with the following significant experimental facts :

(*a*) Oxygen is liberated when tetralin hydroperoxide decomposes; it becomes approximately equivalent to the tetralol produced in the rapid, metal-salt catalysed, decomposition.

(*b*) When the autoxidation is studied by flow methods, the residual off-gases always contain some oxygen, even when the most active catalysts are used and there is prolonged contact between gas and liquid.

(*c*) α-Tetralol is always one of the *major* final reaction products, and is itself too stable to influence appreciably the rate of oxygen uptake by the reacting system (Robertson and Waters, *Trans. Faraday Soc., loc. cit.*).

We therefore conclude that in the steady state (*B*) of the autoxidation the *main* reaction cycle can be represented adequately by the equations

Chain starting.

(3) $\mathrm{R''CH_2} + \cdot\mathrm{OH} \xrightarrow{k_3} \mathrm{R''CH\cdot} + \mathrm{H_2O}$

Long chain sequence.

(1) $\mathrm{R''CH\cdot} + \mathrm{O_2} \xrightarrow{k_1} \mathrm{R''CH{-}O{-}O\cdot}$

(2) $\mathrm{R''CH{-}O{-}O\cdot} + \mathrm{R''CH_2} \xrightarrow{k_2} \mathrm{R''CH{-}O{-}O{-}H} + \mathrm{R''CH\cdot}$

Chain-breaking process (except at very low oxygen pressures).

(5) $\mathrm{R''CH{-}O{-}O\cdot} + \cdot\mathrm{OH} \xrightarrow{k_{tB}} \mathrm{R''CH{-}OH} + \mathrm{O_2}$

This sequence, and the kinetic expression (**E**) to which it leads, can hold so long as an adequate supply of hydroxyl radicals can be maintained by the decomposition of the tetralin hydroperoxide. The requisite condition for this is that there must be so much peroxide within

the system that the rate of production of free hydroxyl (k_c[P]) is *greater than* the combined rates of removal of hydroxyl radicals by both processes (3) and (5).

The excess of free hydroxyl radicals can, of course, be removed by subsidiary oxidation processes which do not affect the course of the main autoxidation cycle (see Part I), as, for example, by oxidations of tetralol or tetralone. Consequently, we find that as the autoxidation of tetralin proceeds in stage (*B*) the fraction of absorbed oxygen which is converted into tetralin hydroperoxide falls progressively. It may be noted that the velocity constant, k_3, of reaction (3) measures the mean life of free hydroxyl radicals in tetralin. The frequency of chain starting is thus limited by the extent to which bimolecular reactions of free hydroxyl, of which (5) is but one, limit its concentration. The final decline in the autoxidation rate [stage (*C*)] may in part be due to the accumulation in the liquid of secondary reaction products which remove hydroxyl radicals.

In the main, however, inhibition of autoxidation seems to be due to the removal of the radicals R″CH· and R″CH–O–O· which are concerned in the main oxygen-uptake cycle, since the partial removal of hydroxyl radicals would not seriously change the overall reaction velocity. Chain stopping by the removal of peroxide radicals, R″CH–O–O· (reaction vii), has now been established conclusively by Bolland and Ten Have (*Trans. Faraday Soc.*, 1947, **43**, 201; Faraday Society Discussion, 1947, **2**, in the press) for the autoxidation of ethyl linoleate in the presence of inhibitors such as quinol, and, for the sequence of reactions (3), (1), (2), (vii), we can derive the relationship

$$+\frac{d[P]}{dt} = k_2\frac{k_3}{k_{tI}}[RH]^2\frac{k_c[P]}{[I]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (F)$$

where k_{tI} is the velocity constant for chain-stopping by the inhibitor; this still denotes reaction at a rate proportional to the square of the hydrocarbon content of the liquid, as found by George and Robertson in these circumstances.

The actual chain-stopping reaction in the presence of organic inhibitors can probably be represented as

$$\text{(vii, } a\text{)} \quad R\text{–}O\text{–}O\cdot + H\text{–}X \longrightarrow R\text{–}O\text{–}O\text{–}H + X\cdot$$

giving a hydroperoxide molecule and a resonance-stabilised radical such as a semi-quinone, particularly since most inhibitors do not destroy hydroperoxide molecules (compare Ivanov, *Compt. rend. Acad. Sci. U.R.S.S.*, 1939, **25**, 34). On the other hand, substances such as iodine, some metallic radicals, and surfaces would more probably act by promoting oxygen evolution, *e.g.*

$$\text{(vii, } b\text{)} \quad R\text{–}O\text{–}O\cdot + \cdot I \longrightarrow R\text{–}I + O_2$$

However, it may be noted that since the main oxygen uptake cycle (reactions 1 and 2) is a *long chain* process, it follows that, approximately, $[R_1]/[R_2] = k_2[RH]/k_1[O_2]$, and thus for a given substance (k_2/k_1 constant) the life of the free hydrocarbon radical will become relatively longer, and that of the peroxide radical R_2 relatively shorter, the lower the oxygen pressure. Consequently, at very low oxygen pressures chain-ending reactions of types (i), (iv), and (vi) will become prominent, and then the autoxidation rate will continue to be dependent upon the partial pressure of the oxygen, as in the initial autocatalytic stage of the reaction. If both the chain-starting and the chain-stopping reactions involve the hydrocarbon radicals, R_1, then the steady state equation (**D**) (p. 1589) may hold. An example of this is afforded by the catalysis of autoxidation by dibenzoyl peroxide for which we may write

Chain starting : $Ph\cdot + R''CH_2 \longrightarrow Ph\text{–}H + R''CH\cdot$

Chain stopping : $Ph\cdot + R''CH\cdot \longrightarrow Ph\text{–}CHR''$

This review of the kinetics of oxygen uptake in the autoxidation of tetralin does not even yet give a complete picture of the subject, for we are still ignorant of the precise fate of the alloxy-radicals derived from the decomposition of the tetralin hydroperoxide and cannot be sure of the extent to which they affect the oxygen-uptake cycle. We do claim to have shown, however, that the free-radical reaction-chain theory will account for all the salient experimental facts, and would emphasize the point that the exact form of the reaction velocity equation seems to be much more dependent upon the nature of the chain-stopping process than upon the chain-starting process.

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320. *Studies on Biological Methylation. Part XII.* A Precursor of the Dimethyl Sulphide evolved by* Polysiphonia fastigiata. *Dimethyl-2-carboxyethylsulphonium Hydroxide and its Salts.*

By Frederick Challenger and Margaret Isabel Simpson.

On exposure to air the marine algæ *Polysiphonia fastigiata* and *P. nigrescens* evolve dimethyl sulphide. An alcoholic extract of *P. fastigiata* on concentration under diminished pressure finally yielded a brown solid. This, with cold sodium hydroxide, evolved dimethyl sulphide, characterised as the mercurichloride. With ammonium reineckate a precipitate was obtained which was converted successively through a crude chloride into the platinichloride, picrate, and a pure sulphonium chloride. This was optically inactive and evolved dimethyl sulphide with cold sodium hydroxide. By direct comparison of this chloride and various derivatives with synthetic specimens it was shown to be *dimethyl-2-carboxyethylsulphonium chloride*. This establishes for the first time the occurrence of sulphonium compounds in plants. The evolution of dimethyl sulphide from the alga probably depends on the enzymic decomposition of this compound or of a more complex derivative.

It was shown by Haas (*Biochem. J.*, 1935, **29**, 298) that the marine algæ *Polysiphonia fastigiata* and *P. nigrescens* when exposed to air evolve dimethyl sulphide. This was characterised as the dibromide, m. p. 93—94°, the mercurichloride, and the platinous chloride compound by aspiration through the appropriate reagents.

It appeared unlikely that the dimethyl sulphide was stored as such in the alga, and the possibility of its occurrence as a sulphonium compound suggested itself. Dr. Haas kindly allowed us to continue the investigation in order to identify the precursor from which it appeared probable (see below) that the sulphide was eliminated by enzyme action. Haas (*loc. cit.*) considered it unlikely that bacterial action was involved in view of the short interval elapsing between the time of collection of the weed and the appearance of the odour. Professor L. Newton of the Department of Botany, University College of Wales, Aberystwyth, has provided us with a regular supply of *P. fastigiata*. This collaboration on her part has been invaluable and we wish to record our sincere thanks. The alga, when received, was still attached to its host *Ascophyllum nodosum*, but was removed before investigation.

When the volatile products from the alga are aspirated through (*a*) mercuric cyanide and (*b*) mercuric chloride, no precipitate is formed in (*a*), indicating the absence of methylthiol, a conclusion in agreement with the relatively pleasant odour which is observed. The properties of the deposit in (*b*) show it to consist solely of the mercurichloride of dimethyl sulphide; mercurated fission products of dimethyl disulphide are absent (see p. 1593; and Challenger and Rawlings, *J.*, 1937, 870; and Blackburn and Challenger, *J.*, 1939, 1872). The volatile sulphur compound is, therefore, homogeneous. The dimethyl sulphide has now been further characterised as benzyldimethylsulphonium picrate. Addition of cold sodium hydroxide to the alga accelerates the elimination of dimethyl sulphide.

The question arises whether dimethyl sulphide evolution is an essential life-process of the alga or whether it is a sign of death or damage. The well-known occurrence of trimethylamine, probably arising from choline, in the living flowers of many trees and plants may be cited.

Two experiments carried out by one of us (M. I. S.) in the marine laboratory at Robin Hood's Bay, through the courtesy of Professor E. A. Spaul, showed that when the alga, still attached to its host, is removed from the rock-pools and immediately re-immersed in a jar of sea-water, no dimethyl sulphide is evolved for at least 24 hours, though after this time a very slow production occurs, possibly due to gradual damage under abnormal conditions. On the other hand the alga, detached from its host and immersed in tap or distilled water, evolves the sulphide in a few hours. When in the rock-pools the alga has no sulphide odour. The production of dimethyl sulphide would seem, therefore, to be an abnormal phenomenon. Further work along these lines is in progress.

Several observations suggest that an enzyme is concerned with the biological process. Grinding the weed with sand or immersing it in distilled water, whereby the cells are ruptured, facilitates the evolution. Haas (*loc. cit.*) states that immersion in boiling water inhibits the production of the odour. We find that weed so treated and allowed to cool evolves no dimethyl sulphide in an air stream (no formation of mercurichloride, or only a trace) but that prolonged boiling with water or addition of sodium hydroxide (purely chemical hydrolysis) causes a fresh evolution. If the alga is left in alcohol for a few hours, and then suspended in water, air removes no dimethyl sulphide but addition of alkali causes a ready evolution.

* Part XI appears in *Biochem. J.*, 1948, **43**, 78.

In all experiments the use of a stream of nitrogen and air-free water made no difference to the result.

The alga exerts no methylating action on sodium arsenite, sodium selenite, or potassium tellurite when suspended in artificial sea-water containing 0·2 and 0·5% of these compounds. The dimethyl sulphide contained no trace of trimethylarsine, dimethyl selenide, or dimethyl telluride. When 1% and 10% sodium ethyl sulphate or 2% triethylsulphonium iodide was used in the "sea-water", methyl ethyl or diethyl sulphides were absent from the dimethyl sulphide.

The procedure leading to the identification of the precursor of the dimethyl sulphide (or its most important fragment) may now be outlined.

The red alga was left in absolute alcohol for 2—3 weeks, and the extract concentrated under diminished pressure to a syrup which yielded a brown solid on rubbing with alcohol. This, with cold sodium hydroxide, evolved dimethyl sulphide, characterised as the mercurichloride $2Me_2S,3HgCl_2$ (Blackburn and Challenger, *J.*, 1938, 1878; Challenger and Charlton, *J.*, 1947, 426). The solid gave a precipitate with aqueous ammonium reineckate, $NH_4[Cr(NH_3)_2(SCN)_4]$. The reineckate ion was removed by silver sulphate, and the sulphate with barium chloride (Kapfhammer and Bischoff, *Z. physiol. Chem.*, 1930, **191**, 179). Evaporation gave a residue which was converted into the platinichloride. This, with metallic silver (Dudley, *Biochem. J.*, 1929, **23**, 1071), gave silver chloride and platinum, and on evaporation as before, a solid chloride. This yielded a picrate which was carefully purified by recrystallisation from alcohol. Decomposition with hydrochloric acid finally yielded a *chloride*, $C_5H_{11}O_2ClS$. This was optically inactive, evolved dimethyl sulphide with cold sodium hydroxide, and was again converted into the *picrate* and also into the bromide, chloroplatinate, and *styphnate*.

The acidity of the chloride and the absence of reaction with 2 : 4-dinitrophenylhydrazine exclude such constitutions as $Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CH_2\cdot CO\cdot CH_2\cdot OH$ or $Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CH(OH)\cdot CH_2\cdot CHO$.

Two structural formulæ appeared probable according to which the chloride would be either an α- or a β-thetin derivative (I) or (II) (see Brown and Letts, *Trans. Roy. Soc. Edinburgh*, 1877—1878, **28**, II, 571).

$Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CHMe\cdot CO_2H$ (I.)

$Me_2\overset{+}{S}(\overset{-}{Cl})\cdot CH_2\cdot CH_2\cdot CO_2H$ (II.)

The corresponding bromides (Carrara, *Gazzetta*, 1893, **23**, 502, 506; Carrara and Rossi, *Atti R. Accad. Lincei*, 1893, **6**, II, 211; Biilmann and Jensen, *Bull. Soc. chim.*, 1936, **3**, 2306) were prepared and converted into the chlorides, picrates, styphnates, and platinichlorides. Mixed m. p. determinations carried out with these compounds and with the chloride from the alga and its corresponding derivatives left no doubt that the algal product is the chloride of the β-propiothetin derivative, dimethyl-2-carboxyethylsulphonium chloride (II).

Holmberg (*Arkiv Kemi, Min. Geol.*, 1946, **21** *B*, No. 7, 1) states that β-methylthiopropionic acid, $SMe\cdot CH_2\cdot CH_2\cdot CO_2H$ with 2½ equivs. of dimethyl sulphate in cold alkaline solution eliminates dimethyl sulphide giving sodium acrylate, characterised as the addition product with benzylsulphinic acid, $Ph\cdot CH_2\cdot SO_2\cdot CH_2\cdot CH_2\cdot CO_2H$. He observed the same reaction with the free dimethyl-2-carboxyethylsulphonium bromide. A similar instability was noticed with benzylmethyl-2-carboxyethylsulphonium bromide, $CH_2Ph\cdot\overset{+}{S}Me(\overset{-}{Br})\cdot CH_2\cdot CH_2\cdot CO_2H$, and cold alkali.

With sodium hydroxide and toluene-*p*-sulphinic acid we find that the synthetic β-thetin bromide (II), the chloride from the alga, and authentic acrylic acid give the same product, $CH_3\cdot C_6H_4\cdot SO_2\cdot CH_2\cdot CH_2\cdot CO_2H$, p-*tolyl-2-carboxyethyl sulphone*.

The α-thetin bromide is more stable and only evolves dimethyl sulphide when heated with sodium hydroxide.

Some interesting decompositions of substituted dimethylethylsulphonium compounds are described by Crane and Rydon (*J.*, 1947, 766). Dimethyl-2-benzoyloxyethylsulphonium iodide, $Ph\cdot CO\cdot O\cdot CH_2\cdot CH_2\cdot\overset{+}{S}Me_2\}\overset{-}{I}$, and $Ph\cdot O\cdot CH_2\cdot CH_2\cdot\overset{+}{S}Me_2\}\overset{-}{I}$ yield dimethyl sulphide, acetylene, sodium iodide, and sodium benzoate and phenoxide respectively with 10% sodium hydroxide at 100°, but in each case recognisable decomposition occurs in M/100-solution at 25° and pH 10.

It is possible that the carboxysulphonium chloride isolated from the alga arises from a more complex derivative, which, during the process of separation, may undergo hydrolysis. The carboxyl group may, in the living alga, exist as a peptide or ester linkage and the anion may be a polysaccharide sulphate ion. Such sulphuric esters occur in many algæ including *P. fastigiata*

(Haas and Russell-Wells, *Biochem. J.*, **1923**, **17**, 696) as well as in agar (Neuberg and Ohle, *Biochem. Z.*, **1921**, **125**, 311). Haas (*Biochem. J.*, **1931**, **25**, 1472) isolated a polypeptide of glutamic acid from *P. fastigiata*.

Hydrolysis of such a linkage may have occurred during the evaporation of the alcoholic extract. This was slightly acid, probably owing to hydrolysis of a potassium or calcium salt of a polysaccharide sulphuric acid.

Only one other instance of the isolation of a sulphonium compound from a natural product is recorded. Neuberg and Grosser (*Centr. Physiol.*, **1905—1906**, **19**, 316) state that the precursor of the diethyl sulphide evolved on warming the urine of dogs with alkali (Abel, *Z. physiol. Chem.*, **1894**, **20**, 253; Christomanos, *ibid.*, **1931**, **198**, 185; **1933**, **217**, 177; *Klin. Woch.*, **1932**, **11**, 177; Wohlgemuth, *Z. physiol. Chem.*, **1933**, **221**, 207) is methyldiethylsulphonium hydroxide, which was isolated as a salt by successive precipitation with phosphotungstic acid and potassium bismuth iodide. They also consider that this hydroxide is formed on feeding diethyl sulphide to dogs. Experimental details are very scanty and no analyses are given.

It is difficult to regard a methyldiethylsulphonium salt as the parent of the diethyl sulphide, for we find that the iodide $\overset{+}{S}MeEt_2\bar{I}$ with sodium hydroxide at 100°, but not below this temperature, gives methyl ethyl sulphide, characterised as the mercurichloride. Moreover, it is clear from Abel's paper (*loc. cit.*) that dogs' urine evolves diethyl sulphide at room temperature with calcium or sodium hydroxide and Christomanos (*loc. cit.*) employed only 60°. The nature of the sulphur compound in dogs' urine is now under investigation in these laboratories. We also find that dimethylethylsulphonium iodide gives dimethyl sulphide with alkali.

Allicin from garlic cloves (Cavallito *et al.*, *J. Amer. Chem. Soc.*, **1944**, **66**, 1950, 1952; **1945**, **67**, 1032), probably $C_3H_5{\cdot}S{\cdot}S{\cdot}C_3H_5$, may perhaps be regarded as of sulphonium type.

$$\overset{\downarrow}{O}$$

Toennies (*J. Biol. Chem.*, **1940**, **132**, 455) and Toennies and Kolb (*J. Amer. Chem. Soc.*, **1945**, **67**, 849) suggested that sulphonium compounds, *e.g.*, derivatives of methionine, may be involved in biological processes, *e.g.*, in the conversion of methionine into cystine (see also Binkley, Anslow, and du Vigneaud, *J. Biol. Chem.*, **1942**, **143**, 59; Binkley, *ibid.*, **1944**, **155**, 39).

The possibility that transmethylation from methionine may involve the intermediate formation of a sulphonium compound was suggested by du Vigneaud *et al.* (*ibid.*, **1941**, **140**, 640; see also Bennett, *ibid.*, **1941**, **141**, 573, and Handler and Bertheim, *ibid.*, **1943**, **150**, 335).

One of us and Charlton (*J.*, **1947**, 424) have shown that in cultures of *S. brevicaulis*, *dl*-methionine gives methylthiol and (presumably by further methylation) dimethyl sulphide. Apparently this fission does not involve the preliminary formation of a sulphonium salt because *dl*-methionine methiodide in cultures of *S. brevicaulis* evolves dimethyl sulphide and no trace of methylthiol.

The behaviour of various sulphonium compounds in mould cultures is being studied by Mr. Y. Cheng Lin, who finds that dimethyl-2-carboxyethylsulphonium chloride (II) readily evolves dimethyl sulphide in bread cultures of *Penicillium notatum* but much less readily with *S. brevicaulis*.

Added August 27th, **1948**.—The biological importance of sulphonium compounds is emphasised by the recent announcement that dimethyl-2-carboxyethylsulphonium chloride and also dimethylcarboxymethylsulphonium chloride (dimethylthetin chloride), $(CH_3)_2\overset{+}{S}(\bar{Cl}){\cdot}CH_2{\cdot}CO_2H$, possess a mobile methyl group, and can support the growth of rats on a diet containing no choline or methionine but containing homocystine (Maw and du Vigneaud, *J. Biol. Chem.*, **1948**, **174**, 381; du Vigneaud, Moyer, and Chandler, *ibid.*, p. 477). In the case of the second thetin which has not yet been discovered in Nature, earlier work (du Vigneaud, "Harvey Lectures," **1942—43**, **38**, 57) had led to the same conclusion. Dubnoff and Borsook (*Fed. Proc.*, **1948**, **7**, 152) have shown that dimethylthetin chloride can methylate homocystine *in vitro*, using a liver preparation.

Experimental.

Further Characterisation of the Dimethyl Sulphide liberated from P. fastigiata. *Absence of Methylthiol and Dimethyl Disulphide.*—The alga was plucked from its host, suspended in water, and a stream of air passed through into two absorption bottles containing 4% mercuric cyanide and two or more containing 3% mercuric chloride. In numerous experiments with different batches of alga no precipitate was ever observed in the mercuric cyanide, indicating the absence of an alkylthiol. The deposit in the mercuric chloride always melted at 156—158°, not depressing the m. p. of an authentic specimen of the mercurichloride of dimethyl sulphide. Its identity was further confirmed by treating a quantity accumulated from several experiments with sodium hydroxide. The liberated dimethyl sulphide was

(*a*) extracted with ether, which was then treated with aqueous potassium chloroplatinite, and (*b*) aspirated through alcoholic benzyl chloride. In (*a*) the solid which separated melted at 160° after two crystallisations. Enebuske (*J. pr. Chem.*, 1888, **38**, 358) gives m. p. 159°, and Challenger, Taylor, and Taylor (*J.*, 1942, 48) give m. p. 160°, for the compound $SMe_2,PtCl_2$. From (*b*), benzyldimethylsulphonium picrate, m. p. 134°, was obtained (Baker and Moffitt, *J.*, 1930, 1722, and Challenger and Charlton, *J.*, 1947, 427, give m. p. 134°). The corresponding *styphnate*, m. p. 114°, was also prepared (Found: C, 45·4; H, 3·85; N, 11·1; S, 8·2. $C_{15}H_{15}O_8N_3S$ requires C, 45·3; H, 3·8; N, 10·6; S, 8·1%).

It remained to be shown that no disulphide, *e.g.*, dimethyl disulphide, arising from the possible, though improbable, complete oxidation of traces of methylthiol was present in the dimethyl sulphide. A further quantity of the dimethyl sulphide mercurichloride, m. p. 157° (from the alga), was recrystallised five times from benzene. It was completely soluble each time and the m. p.s were 156—157°, 156—157°, 155°, 157°, and 156—157°. The compounds SMe·HgCl and $SMe\cdot HgCl,xHgCl_2$ which arise from dimethyl disulphide and mercuric chloride (Blackburn and Challenger, *J.*, 1938, 1872) are very insoluble. The first does not melt below 260° and the second has m. p. 151°. The absence of both these compounds was conclusively proved by treating the mercurichloride from the alga with diluted hydrochloric acid (1 : 2) and aspirating through mercuric cyanide and chloride as before. The dimethyl sulphide was readily evolved in the cold, the cyanide remaining quite clear even on heating the mixture to 60°. At the conclusion of the experiment a small quantity of authentic methylthiomercuric chloride mercurichloride, $SMe\cdot HgCl,xHgCl_2$, was added to the acid reaction mixture. At 60° methylthiol was evolved and a precipitate appeared in the mercuric cyanide.

Preparation of an Extract of the Seaweed.—The *Polysiphonia fastigiata* was picked from the *Ascophyllum nodosum*, washed with water, and dried as thoroughly as possible with towels. It was then left in cold absolute alcohol for periods which varied from 2 to 3 weeks, the alcohol decanted, and a fresh quantity added. After about three extractions the weed was found to evolve hardly any dimethyl sulphide on treatment with cold sodium hydroxide.

The united alcoholic extracts were then filtered and evaporated at 40—50°/15—25 mm. The greenish-brown semi-solid residue tended to crystallise slowly in a desiccator, but was usually triturated at once with absolute alcohol, giving a pale brown solid (P.B.S.). This was acid to litmus and contained organic and inorganic matter, nitrogen, sulphur, and chlorine. Chloride and sulphate ions were present, and the sulphate increased on warming with dilute hydrochloric acid. Warming with dilute sulphuric acid, followed by neutralisation, treatment with solid sodium acetate, glacial acetic acid, and phenylhydrazine, gave an osazone or phenylhydrazone, m. p. 192°, which has not been examined further. As would be expected, the P.B.S. gave a positive Molisch reaction for carbohydrate; the biuret reaction was negative. Amino-acids were present, however, since addition of a dilute solution of triketoindane hydrate ("ninhydrin") to the neutralised P.B.S. gave a violet colour on warming. Ferric chloride gave a green coloration. (The original alcohol washings from the P.B.S. gave this reaction much more intensely and on evaporation gave a viscous residue similar in properties to the P.B.S. but containing much green colouring matter.)

On heating the dry P.B.S., ammonia and dimethyl sulphide were evolved. On treatment with cold sodium hydroxide and aspiration through aqueous mercuric chloride dimethyl sulphide mercurichloride, m. p. and mixed m. p. 157—158°, was obtained. When evolution of this ceased, the alkaline solution was gently warmed, and the vapours absorbed in dilute hydrochloric acid. Evaporation gave a non-deliquescent residue of ammonium chloride. Alkylamines were apparently absent.

On the assumption that it contained a sulphonium compound, concentrated solutions of various salts and acids were added to the P.B.S. in the hope of obtaining a solid derivative. No success was obtained with the sodium salts of styphnic, 2 : 4-dinitrobenzoic, 2 : 4 : 6-trinitrobenzoic, picrolonic, and α-naphthalenesulphonic acids or with potassium ferro- and ferri-cyanides, potassium bismuth iodide, ferric chloride in hydrochloric acid, and ammonium stannichloride in hydrochloric acid. Sodium picrate gave only a precipitate of potassium picrate, but with ammonium reineckate and phosphotungstic acid precipitates were obtained.

Examination of the Reineckate and Phosphotungstate obtained from the P.B.S.—These evolved dimethyl sulphide (characterised as before) on treatment with cold sodium hydroxide or on warming with water and lead hydroxide or baryta. Removal of lead and baryta from the filtered test portions left solutions which, on concentration, contained traces of nitrogen, but no sulphur. They were free from carbohydrate and protein (biuret reaction) but gave a positive ninhydrin reaction. Acidification gave a sharp acidic odour later shown to be due to acrylic acid (see p. 1595). Acidified permanganate was readily reduced in the cold. Ferric chloride gave no colour.

Conversion of the Sulphonium Reineckate into a Sulphonium Platinichloride.—The reineckate (50 g.) was suspended in 50% aqueous acetone (about 100 c.c.) and stirred while excess of saturated (N/25) aqueous silver sulphate solution was added from a burette. On complete precipitation of the reineckate as silver salt, the filtered solution was treated with N/12-barium chloride solution, in amount equivalent to the silver sulphate added. The liquid was filtered through a sintered-glass crucible, and the filtrate (acid to litmus) evaporated to dryness under diminished pressure at 50—60°, leaving a brownish viscous residue which showed signs of solidifying when kept in a desiccator. It evolved dimethyl sulphide with cold aqueous sodium hydroxide. On addition of a very concentrated aqueous solution of chloroplatinic acid, a platinichloride separated ou rubbing and standing for a few minutes. This was separated and washed with successive small quantities of water–alcohol (30 : 70). The aqueous filtrate gave a further deposit on addition of alcohol. The platinichloride was recrystallised from water–alcohol (1 : 10), and then decomposed between 194° and 202° according to the rate of heating. It gave a negative Molisch reaction and evolved dimethyl sulphide with cold alkali or when heated alone.

Conversion of the Sulphonium Platinichloride into the Picrate.—The platinichloride, dissolved in the minimum amount of water, was treated with twice its weight of silver, prepared by Dudley's method (*loc. cit.*). (The silver had been very carefully washed, otherwise it was found to occlude ferrous sulphate which then contaminated the picrate.) The mixture was shaken vigorously for a few minutes until the solution was colourless. Silver, platinum, and silver chloride were separated, leaving a solution of the

sulphonium chloride, which was evaporated to dryness at 50—60° under diminished pressure. Addition of saturated aqueous sodium picrate to the residue gave, after rubbing with a glass rod and standing for a few minutes, a yellow crystalline precipitate. This was separated, washed with absolute alcohol, and dried in a desiccator; m. p. 128°. After recrystallisations from the same solvent the m. p. was 131° (decomp. 131·5°). On three further crystallisations the m. p. was on each occasion 131·5° (decomp. 132°). The m. p.s of these five specimens were taken simultaneously on the same thermometer. The picrate readily evolved dimethyl sulphide with cold sodium hydroxide.

In later experiments the picrate was prepared from the chloride resulting directly from the removal of the reineckate ion, without passing through the platinichloride stage. The product was at first somewhat discoloured but on recrystallisation from alcohol the pure picrate was obtained.

Conversion of the Sulphonium Picrate into the Chloride.—The picrate, three times recrystallised, was weighed, dissolved in 1 equiv. of dilute hydrochloric acid, and shaken repeatedly with successive quantities of ether till the extract was colourless. The aqueous solution was then faintly red and still acidic. Evaporation at 50—60° under diminished pressure gave a residue which gradually crystallised in almost colourless needles when left in a vacuum desiccator. Crystallisation from absolute alcohol gave colourless needles of constant m. p. 134° (decomp.). These contained no nitrogen and gave no Molisch reaction before or after removal of dimethyl sulphide with alkali. On exposure to air for a few minutes the m. p. quickly fell to below this value as the chloride is deliquescent (Found, by micro-analysis: C, 35·5, 35·7; H, 6·6, 6·45; Cl, 20·3, 20·6; S, 18·9, 19·0. Calc. for $C_5H_{11}O_2ClS$: C, 35·2; H, 6·5; Cl, 20·7; S, 18·7%). The compound contained ionic chlorine and was acid to litmus. This and the earlier experiments suggested the formula $\overset{+}{S}Me_2(\bar{Cl})\cdot C_2H_4\cdot CO_2H$. The carboxyl group and the ionised chlorine were determined volumetrically.

The chloride (1·1308 g.) in 100 c.c. of water was titrated in 10-c.c. portions with 0·036N-sodium hydroxide, using phenolphthalein. When the carboxyl group was neutralised the indicator turned but the colour was not permanent, for the salt began to decompose thus: $\overset{+}{S}Me_2(\bar{Cl})\cdot C_2H_4\cdot CO_2Na + NaOH = SMe_2 + C_2H_3\cdot CO_2Na + NaCl + H_2O$, and an odour of dimethyl sulphide was detected at the end-point. Serious error was avoided by shaking thoroughly during the titration and taking the reading when the pink colour was first produced (Found: CO_2H, 25·7%; equiv., 172).

The chloride ion in the titrated solutions was determined by Volhard's method (Found: Cl, 20·6). An earlier specimen of the sulphonium chloride gave by gravimetric analysis Cl, 20·5 (Calc. for $C_5H_{11}O_2ClS$: CO_2H, 26·3; Cl, 20·7%; equiv., 170·5).

The platinichloride, prepared from the pure chloride, melted at 193° or 200° according to the rate of heating. Carrara and Rossi (*loc. cit.*) give m. p. 184° (see also Billows, *Gazzetta*, 1893, **23**, I, 508) [Found: Pt, 29·5. Calc. for $(C_5H_{11}O_2ClS)_2PtCl_4$: Pt, 28·8%].

Properties of the Sulphonium Chloride: Reconversion into Picrate.—The chloride liberated carbon dioxide from sodium hydrogen carbonate and was optically inactive in water (*c*, 2·5, 5, or 10). When it was heated above its m. p., dimethyl sulphide (mercurichloride, m. p. 157—158°) and some hydrogen chloride were obtained. The residue had a sharp acidic odour (see below) and decolorised bromine water and potassium permanganate. It gave no colour with ferric chloride and no precipitate with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid.

As stated on p. 1592, these results indicate that the chloride is dimethyl-2-carboxyethylsulphonium chloride. It could be reconverted into the picrate of unchanged m. p. 131·5° (decomp. 132°). Specimens of the *picrate*, obtained from the chloride, with or without passing through the platinichloride, were analysed (Found: C, 36·8; 36·5; H, 3·65, 3·75; N, 11·3. $C_{11}H_{13}O_9N_3S$ requires C, 36·4; H, 3·6; N, 11·6%). Subtraction of the picrate ion $C_6H_2O_7N_3$ gives for the sulphonium cation $C_5H_{11}O_2S$, in agreement with the formula of the chloride $C_5H_{11}O_2ClS$. Determination of the picrate ion as nitron picrate gave for the molecular weight of the sulphonium ion 136·1 ($C_5H_{11}O_2S$ requires *M*, 135. The *styphnate*, crystallised from alcohol, had m. p. 148° (Found: C, 35·1; H, 3·4; N, 10·7. $C_{11}H_{13}O_{10}N_3S$ requires C, 34·8; H, 3·45; N, 11·1%).

The sulphonium bromide was prepared by repeated evaporation of the algal chloride with hydrobromic acid almost to dryness at 100° during 4—5 hours. The final residue solidified on treatment with absolute alcohol, from which it was recrystallised by addition of ether. It melted at 112—114°. Carrara and Rossi (*loc. cit.*) give m. p. 115°.

Decomposition of the Sulphonium Chloride with Sodium Hydroxide.—The algal chloride (1 g.) was treated with excess of sodium hydroxide, and the liberated dimethyl sulphide removed by a stream of air at room temperature. To the odourless solution toluene-*p*-sulphinic acid (1 g.) was added, and the mixture slightly acidified with hydrochloric acid. A white solid separated on standing which, after recrystallisation from water and drying in a desiccator, had m. p. 112°. A synthetic specimen of dimethyl-2-carboxyethylsulphonium chloride (see p. 1596) on similar treatment gave a product of m. p. 112—113° and mixed m. p. 112—113° with the algal product.

The same compound, *p-tolyl 2-carboxyethyl sulphone*, was prepared from acrylic acid and the equivalent amount of toluene-*p*-sulphinic acid in dilute alkali. Acidification as before gave a product which, when crystallised from water and thoroughly dried in a desiccator, had m. p. and mixed m. p. 112° with the two specimens just described prepared from the algal and synthetic chlorides (Found: C, 52·0; H, 5·2. $C_{10}H_{12}O_4S$ requires C, 52·5; H, 5·25%).

For comparison, the m. p.s of toluene-*p*-sulphinic acid and its possible transformation products toluene-*p*-sulphonic acid and *p*-tolyl disulphoxide, $C_7H_7\cdot SO_2\cdot S\cdot C_7H_7$, are 90°, 92° and 104·5° (hydrated and anhydrous respectively), and 76°, respectively.

Preparation of Reference Compounds.—Dimethyl-2-carboxyethylsulphonium derivatives. (a) The bromide was prepared by the method of Carrara (*loc. cit.*), but as his procedure is not very clear the following details are given. Equimolecular quantities of β-bromopropionic acid and dimethyl sulphide were mixed. After 24 hours at room temperature a test portion yielded only a small quantity of sulphonium compound, insoluble in ether, giving a precipitate with aqueous ammonium reineckate. The reaction mixture was then refluxed at 55—60° for 6—8 hours. It solidified on cooling; washing

with dry ether removed unchanged reactants, leaving a white solid which was recrystallised from absolute alcohol; m. p. 112—114°. Carrara (*loc. cit.*) gives m. p. 115° (decomp.). Under these conditions β-chloropropionic acid and dimethyl sulphide did not react, but (b) the *chloride* was obtained from the bromide and silver chloride. On recrystallisation from absolute alcohol it melted at 134° (Found: C, 34·7; H, 6·5; Cl, 20·3, 20·6. $C_5H_{11}O_2ClS$ requires C, 35·2; H, 6·45; Cl, 20·7%). When a mixture of equimolecular amounts of β-iodopropionic acid and dimethyl sulphide was left at room temperature only traces of a sulphonium compound could be detected by ammonium reineckate. After a few hours' refluxing a sulphonium compound was isolated as before. This gave no picrate and only evolved dimethyl sulphide on boiling with alkali. It melted at 202—204°, and at 200—202° in admixture with authentic trimethylsulphonium iodide, m. p. 202—204° (all with decomp.). Büilmann and Jensen (*loc. cit.*) obtained trimethylsulphonium bromide from dimethyl sulphide (2 mols.) and α-bromopropionic acid (1 mol.) or its ethyl ester (1 mol.) at 100°. With the acid and sulphide in equimolecular proportions at room temperature the thetin bromide was formed (see also Carrara, *loc. cit.*). Trimethylsulphonium iodide was formed from methyl iodide (2 mols.) and ethyl α-methylthiopropionate at 100°.

(c) The picrate, m. p. 132°, was prepared from the bromide (Found: C, 36·6; H, 3·6; N, 11·7; S, 9·0%).

(d) The styphnate had m. p. 148·5—149°, the m. p.s on recrystallisation from alcohol being 148—148·5°, 149°, 148·5—149°, 148·5—149°, 148·5° (Found: C, 34·9; H, 3·35%).

Dimethyl-1-carboxyethylsulphonium derivatives. (e) The bromide was prepared by Carrara's method (*loc. cit.*) at room temperature. The solid, crystallised from warm alcohol by addition of ether, had m. p. 94—95° and was very deliquescent. Carrara gives m. p. 84—85° (decomp.). (f) It was converted into the picrate. Five recrystallisations from alcohol only raised the m. p. from 151·5—152° to 152—152·5°. (g) The styphnate had m. p. 151—151·5°, the figures on recrystallisation from alcohol being 152°, 150—151°, 151·5—152°, 151—151·5°.

The chloride was prepared from the bromide and silver chloride. It was very deliquescent and was not crystallised. (h) It gave a platinichloride, m. p. 111°, on recrystallisation from absolute alcohol. Billows (*loc. cit.*) gives m. p. 105—106°.

Table of m. p. and mixed m. p. of derivatives of dimethyl-α- and -β-propiothetins.

	Chloride.	Bromide.	Picrate.	Styphnate.	Platinichloride.
α-Thetin deriv.	—	94—95°	152—152·5°	151—151·5°	111°
β-Thetin deriv.	134°	112—114	132	148·5—149	193 or 200 *
Mixed m. p.	—	74	124	139	111—200
Algal product	134	112—113	131·5	148	193 or 200 *
Algal product + α-thetin deriv.; mixed m. p.	—	75	122	138	111—200
Algal product + β-thetin deriv.; mixed m. p.	132—133	112—113	131—132	148	193 or 200 *

* Dependent on the rate of heating.

Preparation of Other Sulphonium Compounds.—(1) *Dimethyl-n-amylsulphonium iodide.* Methyl *n*-amyl sulphide (Blackburn and Challenger, *J.*, 1938, 1875) and its equivalent of methyl iodide were left at room temperature without a solvent for 24 hours. The resulting *iodide* was recrystallised from alcohol–ether; m. p. 102° (Found: I, 48·8. $C_7H_{17}IS$ requires I, 48·8%).

(2) *Dimethyl-2-hydroxyethylsulphonium iodide.* Methyl 2-hydroxyethyl sulphide (*Org. Synth.*, Coll. Vol. II, 346) gave a mercurichloride, m. p. 94°, from alcohol. A mixture of the sulphide and an equivalent of methyl iodide gradually became warm and a solid separated. This was recrystallised from absolute alcohol; m. p. 57—59°. It was extremely deliquescent.

(3) *Dimethyl-2-acetoxyethylsulphonium iodide.* The methyl 2-hydroxyethyl sulphide was refluxed with acetic anhydride for 30 mins., cooled, shaken with aqueous sodium carbonate solution, and the mixture extracted with ether, yielding *2-acetoxyethyl sulphide* as a colourless oil having an odour resembling that of amyl acetate; b. p. 68—70°/15 mm. (Found: C, 44·5; H, 7·6; S, 24·7. $C_5H_{10}O_2S$ requires C, 44·7; H, 7·5; S, 23·8%). The mercurichloride, when recrystallised from alcohol, melted at 93°, and at 85° in admixture with the mercurichloride of the unacetylated sulphide, m. p. 94°.

With methyl iodide in the cold a syrup separated after a day. This was very deliquescent and could not be crystallised. The picrate recrystallised from alcohol, had m. p. 133—134°.

Methyl 2-hydroxyethyl sulphide (2·5 g., 1 mol.), tetra-acetyl glucosidyl bromide (11·0 g.; 1 mol.), and active silver oxide (3·15 g., 0·5 mol.), prepared by the method of Helferich and Klein (*Annalen*, 1926, **450**, 225), were shaken in dry benzene for an hour and heated under reflux for the same time. The mixture darkened, silver and silver bromide were deposited, and an odour resembling that of amyl acetate was observed. Removal of solvent and extraction with water and ether yielded an oil of b. p. 55—60°/15 mm. with the same odour. It gave a mercurichloride of m. p. and mixed m. p. 93° with the mercurichloride of methyl 2-acetoxyethyl sulphide. The attempt to prepare the acetate of a 2-methylthioethyl glucoside was therefore unsuccessful.

Decomposition of Some Sulphonium Compounds by Aqueous Sodium Hydroxide.—Each sulphonium compound was treated with excess of 2N-sodium hydroxide, and the temperature slowly raised in a water-bath. Volatile matter was aspirated into aqueous mercuric chloride. Trimethylsulphonium iodide, m. p. 203—206° (decomp.) (Steinkopf and Muller, *Ber.*, 1923, **56**, 1928, give m. p. 202°, decomp.), dimethylbutylsulphonium iodide, m. p. 81·5° (Ingold *et al.*, *J.*, 1933, 535, give m. p. 82°), and dimethylamylsulphonium iodide (see above) yielded dimethyl sulphide mercurichloride, m. p. 157—158°, with boiling sodium hydroxide, but no action was observed below 100°. Under exactly similar conditions methyldiethyl- and triethyl-sulphonium iodides yielded the mercurichlorides of methyl ethyl sulphide,

m. p. 127°, and diethyl sulphide, m. p. 118° (Challenger and Rawlings, *J.*, 1937, 868, give m. p. 127—128° and 119°, respectively). No change occurred below 100°.

Methylmethioninesulphonium iodide, $\overset{+}{S}Me_2(\bar{I})\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot CO_2H$, evolved dimethyl sulphide with 2N-sodium hydroxide just below the boiling point. No decomposition occurred below 90°. Dimethyl-2-hydroxyethylsulphonium iodide and dimethyl-2-acetoxyethylsulphonium picrate were unchanged at room temperature, but liberated dimethyl sulphide at 55° and 60—70°, respectively. The m. p.s of the mercurichlorides were 156—157°, 157—158°, and 157°.

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321. *The Effect of Metallic Impurities in Magnesium on the Formation of Grignard Compounds.*

By T. J. REID and A. R. UBBELOHDE.

Magnesium alloyed with various amounts of aluminium, copper, and manganese has been used in preparing ethylmagnesium iodide under quantitatively controlled conditions. The percentages of each of three main reactions which take place were determined, *viz.*,

Grignard reaction :

$$C_2H_5I + Mg \longrightarrow C_2H_5\cdot MgI$$

Wurtz reaction :

$$2C_2H_5I + Mg \longrightarrow C_4H_{10} + MgI_2$$

Disproportionation reaction :

$$2C_2H_5I + Mg \longrightarrow C_2H_6 + C_2H_4 + MgI_2$$

Compared with very pure magnesium, alloys containing up to 50% of aluminium do not substantially increase the Wurtz and the disproportionation reaction. Alkylaluminium compounds are formed as well as alkylmagnesium compounds. A 50% Mg : 50% Al alloy has been used successfully in a Grignard synthesis.

Alloys containing up to about 2% of copper or manganese increase the Wurtz and especially the disproportionation reaction very markedly. A preliminary investigation has been made of the mechanisms proposed to explain this increase.

THE effect of metallic impurities on the formation of Grignard compounds is of considerable practical and theoretical interest. From the practical standpoint, it has been reported (Johnson and Adkins, *J. Amer. Chem. Soc.*, 1931, **53**, 1520) that 12% of copper in magnesium lowers the yield of Grignard reagent and increases the combined Wurtz and disproportionation reactions (cf. also Cusa and Kipping, *J. Soc. Chem. Ind.*, 1934, **53**, 213). It was of interest to test whether on reducing impurities down to the limits in a sample of specially pure distilled magnesium (Fe, 0·0004%; Zn, 0·003%; Pb, 0·002%; Si, Ni, Sn, Cd, <0·005%) these side reactions might be completely suppressed.

A further practical point refers to the possible use of alloys of magnesium containing substantial amounts of other metals, such as aluminium, to form mixed alkyl reagents. Apart from economic considerations, this might facilitate the preparation of mixed-metal Grignard reagents of controlled reactivity, which could be used in certain syntheses where more than one reactive grouping is attacked by alkylmagnesium compounds. Although the formation of alkylaluminium halides from pure aluminium is a comparatively sluggish process which has not been much investigated (Hnizda and Kraus, *J. Amer. Chem. Soc.*, 1938, **60**, 2276; Grosse and Mavity, *J. Org. Chem.*, 1940, **5**, 106), the results described below show that when, for example, a 50 : 50 Mg–Al alloy is used, the dissolution of the magnesium in an ethereal solution of alkyl halide appears to leave the aluminium in such a finely divided active state that it likewise dissolves. This opens up the possibility of forming other metal alkyls by using suitable alloys of the metal with magnesium. A synthesis carried out with the magnesium aluminium alkyl mixture is described below.

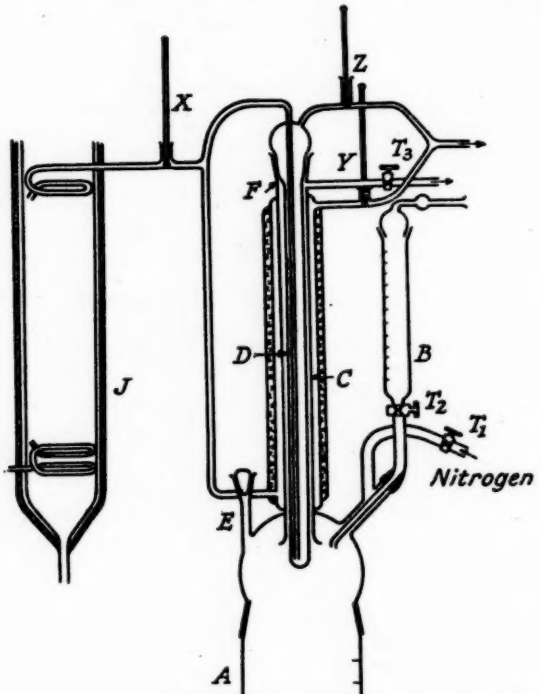
From the theoretical standpoint, the effect of metallic impurities in magnesium is of interest in view of the results obtained by adding halides of the transitional elements to solutions of Grignard reagents (cf. Hey, *Ann. Reports*, 1944, **41**, 195, who gives a number of references). Small amounts of metallic impurities alloyed with the magnesium would be left in a finely divided condition when the magnesium dissolves, and might be expected to form reactive intermediates, similar to those obtained when the corresponding halides are added to the solution of Grignard reagents.

To test this possibility, alloys of magnesium were used which contained up to 2% of manganese and 2·5% of copper, severally. As is described below, these impurities have a substantial influence on the course of the reactions. Experiments were also carried out in which the magnesium was plated with thin films of nickel, copper, iron, and manganese before addition of the alkyl iodide. However, except in an isolated experiment with nickel, metals added in this way were not found to influence the course of the reaction. The significance of this observation is discussed below.

Experimental.

Experimental Details.—The apparatus used (see Fig.) for the preparation incorporated (*a*) a method of drawing off aliquot portions of the Grignard reagent for analysis, (*b*) facilities for the collection of the evolved gases, (*c*) an efficient condenser to condense most of the ether out of the gas before gas analysis.

The reaction vessel *A* has a calibrated scale attached so that the total volume of liquid could be observed with the aid of a cathetometer to ± 0·1 c.c. This vessel is joined to the upright condenser *C*



which has an inner condensing surface *D* fitted inside it by means of the ground-glass join *F*. The gases and ether vapour evolved in the reaction vessel *A* ascend the space between these condensing surfaces, pass out through the Tap *T*3, and are collected over a saturated sodium chloride solution in a gasometer. Water circulating through the condenser is cooled to 3° in the insulated cooler *J*, where it circulates through a copper spiral round which is packed a mixture of ice and salt. This condensation at 3° serves to remove as much ether vapour as possible, without unduly retaining butane. Ethyl iodide is added from the burette *B*. For the purposes of analysis samples of the solution of the Grignard reagent may be drawn off in a pipette through the outlet *E*, using counter-currents of dry oxygen-free nitrogen to protect the contents of the vessel.

Before the preparation was carried out the apparatus was thoroughly cleaned and dried by means of hot air and swilling with pure dry ether. All taps were lubricated with petroleum jelly.

2 G. of magnesium in the form of turnings were rinsed in alcohol and ether and placed in the vessel *A*. 25 C.c. of pure ether and a crystal of iodine were added. Pure dry nitrogen was passed through the apparatus for 15 minutes to expel all the air. A solution of 6·9 g. of ethyl iodide in ether was put in the burette *B*. About 1·5 g. of ethyl iodide were added to the magnesium, and the vessel gently heated till the reaction started. The remaining ethyl iodide was added slowly as recommended by Gilman and Meyers (*J. Amer. Chem. Soc.*, 1923, **45**, 159). When all the ethyl iodide had been added (35 mins.), the reaction mixture was refluxed for 20 minutes to drive over the evolved hydrocarbons. The solution was then diluted to 60 c.c. and again refluxed. A current of nitrogen was then passed to take all the evolved hydrocarbons into the gasometer.

The nitrogen and ether were carefully purified (see Mackle and Ubbelohde, this vol., p. 1161).

Estimation of the Grignard Reaction.—Gilman's acid titration method (*J. Amer. Chem. Soc.*, 1927, **49**, 3181) was used for the estimation of the Grignard reaction, phenolphthalein being used as indicator. Magnesium hydroxide is sufficiently alkaline to give satisfactory titrations, but when aluminium is also present its evaluation by this method is not complete. In this case aluminium and magnesium in the solution were estimated by precipitation with 8-hydroxyquinoline (Vogel, "Quantitative Analysis", 1939).

Estimation of Side Reactions.—The Wurtz and the disproportionation side reaction were estimated by measuring the amount of ethylene, ethane, and butane evolved during the reaction. These hydrocarbons are contained in a gasometer together with ether vapour and nitrogen. The total volume of gas was observed, and a portion analysed in a type of gas analysis apparatus in which the gases were absorbed by "fountains" of the various reagents. The ether vapour was absorbed with 10% sulphuric acid, and the ethylene with bromine water. The ethane and butane were determined simultaneously by sparking with excess of oxygen and measurement.

Ethane and ethylene, formed from the disproportionation reaction, should be evolved in equal volume. In practice there is always rather more ethane than ethylene. In spite of rigorous precautions it was not possible to exclude water altogether, and some hydrolysis of ethylmagnesium iodide occurs during its formation. To avoid confusion from this effect, the amount of disproportionation in Tables I—III was always calculated from the volume of *ethylene* evolved. The volume of butane gives a measure of the amount of Wurtz reaction. The probable error in the gas volumes was $\pm$ 1·5% and in the estimation of Grignard reagents $\pm$ 0·25%.

Analysis of the Sediment from Mg/Mn 98/2.—The black slurry was dissolved in warm concentrated sulphuric acid and diluted. The ether was boiled off, and the solution analysed for iodine with silver nitrate and for manganese by the bismuthate method.

Methods and Results.—Throughout the experiments, ethyl iodide was used as the alkyl halide. It was allowed to react with magnesium in the calibrated vessel *A* (see Fig.). Specially purified ether was used as solvent. During the reaction and also during transfer of ethereal solutions from one vessel to another, all solutions were protected from oxygen and water vapour by an atmosphere of oxygen-free nitrogen (Mackle and Ubbelohde, *loc. cit.*). The contents of the vessel *A* were allowed to settle and aliquot parts of the solution were removed for analysis, so as to evaluate the extent of the Grignard reaction. Gaseous products of reaction passed up the double-surface condenser *C,D* which is specially designed to permit easy cleaning and rigorous drying. They were then collected for gas analysis, so as to evaluate the extent of the Wurtz and disproportionation reactions. Typical results are given in Table I.

TABLE I.

Reactions with pure magnesium.

		Reaction, %.		
Expt.	Mg, g.	Grignard.	Wurtz.	Disproportionation.
A	2·0	90·0	4·4	3·7
B	2·0	90·6	5·9	2·3
C	2·0	90·8	4·15	3·5
D	2·0	90·1	4·2	3·8

In these reactions the magnesium theoretically required by the ethyl iodide used was 1·09 g. Percentage reactions are calculated from the amounts of the various products relative to the total ethyl iodide consumed.

The data illustrate the sort of variation observed under conditions which were kept as constant as possible. Side reactions account for about 8% of the ethyl iodide, and together with the Grignard reaction they account for at least 98% of the ethyl iodide. It is not yet clear whether the remaining 2% is due to further reactions, or to difficulties in the analyses. Experiments made in another connection (Mackle and Ubbelohde, *loc. cit.*) indicate that attack of the ether solvent during the formation of the Grignard reagent can be neglected under the above conditions.

TABLE II.

Reactions with Mg/Al *alloys.*

			Reaction, %.		
Expt.	Alloy.	G.	Grignard (apparent).	Wurtz.	Disproportion-ation.
I	100% Mg	2·0	(See below)	4·2	3·8
A	5% Al	2·1	75·7	4·3	3·4
B	,,	,,	74·3	4·7	4·1
C	,,	,,	75·4	4·9	4·55
D	,,	,,	72·6	4·4	4·0
E	50% Al	4·0	77·0	3·4	1·6
F	,,	—	80·0	3·8	2·5
G	,,	—	76·4	3·15	3·2

In experiments recorded in Table II the total amount of alloy used was such as to contain 2·0 g. of magnesium, to compare with Table I. Comparison of these data with those of Table I shows that the amount of ethyl iodide consumed in side reactions is not increased by alloying the magnesium with aluminium.

The figures in the column under " Grignard (apparent) " do not allow for the simultaneous formation of alkylaluminium compounds. It was found that these do not respond to the Gilman method of acid–alkali analysis. The presence of these aluminium alkyls was, however, verified as follows: (1) Qualitative tests showed that aluminium compounds were formed which were soluble in ether. (2) In special experiments the total magnesium and aluminium in ethereal solution were estimated by using 8-hydroxyquinoline, with the following results, in experiments under similar conditions to those in Tables I and II.

Alloy.	Mg dissolved, g.	Al dissolved, g.
Pure Mg	0·970	—
Mg 95, Al 5	0·839	0·037
Mg 50, Al 50	0·727	0·161

Although the analytical separation is not fully satisfactory owing to various complications, the figures show that the amount of soluble aluminium compounds formed roughly accounts for the ethyl iodide not accounted for in the figures of Table II, having regard to the higher valency of the aluminium. It seems likely that compounds are formed according to the reaction $3C_2H_5I + 2Al \longrightarrow AlIEt_2 + AlI_2Et$, since Hnizda and Kraus (*loc. cit.*) did not observe any triethylaluminium in their experiments. Further investigation is, however, required to establish this point in detail. The fact that the Mg : Al ratio in solution is greater than 50/50 in the above experiments is probably due to the use of excess of alloy (see Table II), with preferential dissolution of the magnesium.

In certain circumstances it may be more economic to use an alloy such as 50/50 Mg/Al in place of pure magnesium. In order to obtain a preliminary idea of the possibilities, two Grignard syntheses were attempted. For these the recommended amount of magnesium was taken (cf. *Org. Synth.*) and the same amount of 50/50 Mg/Al was used in the comparison experiment.

In the preparation of phenylethylcarbinol from benzaldehyde, the yield of crude product was 17·1 g. when pure magnesium was used and 16·8 g. with the 50/50 alloy. In this case the mixed alkyls can be used successfully. On the other hand, in the preparation of triethylcarbinol from ethyl carbonate, no appreciable yield was obtained from the mixed alkyls. The reason for this is under further investigation.

TABLE III.

Reactions with magnesium alloyed with transitional elements.

			Reaction, %.		
Exp.	Alloy.	G. used.	Grignard.	Wurtz.	Disproportionation.
A	0·5% Mn	2·01	75·7	7·7	13·85
B	,,	,,	76·6	6·5	16·45
C	2·0% Mn	2·04	68·8	8·5	23·0
D	,,	,,	66·7	6·9	24·5
E	,,	,,	59·0	10·5	30·35
F	0·5% Cu	2·01	78·8	8·85	11·2
G	,,	,,	80·5	7·65	11·6
H	2·5% Cu	2·05	70·3	11·9	16·2
I	,,	,,	68·7	12·25	17·2
J	,,	,,	68·1	11·2	18·35

In these experiments the amounts of alloy used contained 2·0 g. of magnesium, in order to give data comparable with Tables I and II.

Unlike aluminium, both copper and manganese when alloyed with magnesium increase the side reactions, particularly the disproportionation reaction. Two further items of experimental evidence have a bearing on these observations:

(1) It was of interest to see whether the transitional metals formed any *soluble* alkyl compounds, like those formed from Mg/Al. To economise labour, tests were restricted to the experiments with Mg 98%, Mn 2·0%. During the dissolution of this alloy in ethereal ethyl iodide, a fine black powder filled the vessel. This was allowed to settle, together with any unreacted alloy, before carrying out the usual analyses on the clear supernatant solution. Qualitative tests showed only traces of manganese in this clear solution. The weight of manganese present in the black sediment was almost equal to the weight originally present in the weight of alloy taken. The black sediment also contained very substantial amounts of combined iodine.

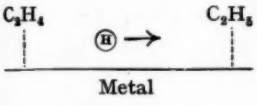
(2) A second line of investigation was to test if layers of metals such as copper and manganese deposited on the outer surface of the magnesium filings, so as to form magnesium–metal couples, produced a similar change in the extent of the side reactions to that when the metals are alloyed with the magnesium. Chips of pure magnesium were washed with aqueous solutions of a salt of copper, manganese, nickel, or iron, severally, until tests showed all the metal had been deposited on the surface of the magnesium. Various degrees of plating were investigated with each metal, using amounts ranging from 0·0005% to 1·0% of the magnesium. The magnesium–metal couples were then washed with ether and placed in ethereal magnesium iodide. Whereas pure magnesium and the various alloys gave induction periods of much the same duration, before dissolving actively in the ethereal ethyl iodide, the plated magnesium chips gave long induction periods except when the plating was very thin. In most cases couples prepared in this way gave reaction ratios very similar to those with pure magnesium. One isolated observation with Mg–Ni couple, with a prolonged induction period, subsequently gave a reaction with 43·5% disproportionation, but the conditions for obtaining this result could not be repeated.

Discussion.

Reference has been made above to the practical significance of this investigation. Brief mention may also be made of some theoretical considerations on the mechanism of the Grignard reaction, and of side reactions.

Two main types of mechanism may be proposed to explain the formation of the Grignard reagent and of the simultaneous occurrence of side reactions. These may be described as the " heterogeneous " and the " homogeneous " mechanism. According to the " heterogeneous " mechanism, the alkyl iodide adsorbed on the magnesium becomes split by activation into ethyl radicals and iodine atoms, which then weaken the attraction of a magnesium atom to the remaining parts of the metal sufficiently to permit its being dissolved as the alkylmagnesium iodide. With less reactive metals such as aluminium, a high temperature is required for effective attack of the surface atoms in this way, unless the aluminium is left in a finely divided state, as in our experiments, by prior dissolution of the magnesium in the alloy.

The essential feature about the heterogeneous mechanism is that the disproportionation reaction would involve a heterogeneous migration of hydrogen atoms from one alkyl radical to a neighbouring alkyl radical. Metals which are effective hydrogenation and dehydrogenation catalysts would be expected to favour the side reaction involving disproportionation, by facilitating such transfers. This would explain the distinction of behaviour between aluminium, on the one hand, and metals such as copper and manganese on the other. Objections to the heterogeneous mechanism are the fact that metal couples were not found to be effective, and that the " heterogeneous " mechanism does not offer any simple explanation of the increased Wurtz reaction due to such impurities.



According to the " homogeneous " mechanism, the impurities in the magnesium dissolve in the first instance in the ether. The molecules produced may perhaps have the structures suggested by Kharasch (Hey, *loc. cit.*) though the evidence does not exclude other soluble intermediates.

The essential feature of the explanation proposed by Kharasch is that free radicals are formed *in solution*, by some such scheme as (in our examples)

$$C_2H_5{\cdot}MgI + MnI_2 \longrightarrow C_2H_5{\cdot}MnI + MgI_2$$
$$2C_2H_5{\cdot}MnI \longrightarrow C_4H_{10} + 2MnI{\cdot}$$
$$MnI{\cdot} + C_2H_5I \longrightarrow C_2H_5{\cdot} + MnI_2$$

The alkyl radical then either disproportionates with other radicals or dimerises. This explanation fits a number of facts, but does not readily accord with the relative increases of the Wurtz and the disproportionation reaction as the amount of the manganese is increased. Further work seems required to decide whether the catalytic effect of the impurities in the magnesium acts in a heterogeneous or a homogeneous manner to increase the side reactions.

Thanks are due to Messrs. Magnesium Metal Corporation for their help in supplying pure magnesium and pure magnesium alloyed with the various metals quoted above.

Queen's University, Belfast. [*Received, November 17th,* 1947.]

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322. 1-*Phenyl-2-naphthyl- and* 1 : 2-*Dinaphthyl-ethylenes.*

By James L. Everett and George A. R. Kon.

The preparation of several diarylethylenes in their stereoisomeric forms is described.

In connection with studies on basically-substituted stilbenes (more strictly, 1 : 2-diarylethylenes) which were found to be carcinogenic and to affect the growth of transplanted tumours in rats (Haddow, Harris, Kon, and Roe, *Phil. Trans.*, 1948, *A*, **241**, 147) certain simple diarylethylenes were required. Since some of these were not known and the data regarding others were not satisfactory, it was decided to prepare them and, if possible, to obtain both stereoisomeric forms.

1-Phenyl-2-(1-naphthyl)ethylene, m. p. 72·5—73·5°, of unknown configuration has been prepared by Balla (*Compt. rend.*, 1934, **198**, 947), whilst Thiele has tentatively ascribed the same structure to a hydrocarbon, m. p. 155° (*Ber.*, 1899, **32**, 1296). Ruggli and Preuss (*Helv. Chim. Acta*, 1941, **24**, 1345) made an unsuccessful attempt to prepare it by decarboxylation of α-phenyl-

β-(1-naphthyl)acrylic acid, which should have yielded the *cis*-compound, by analogy with the numerous examples examined by Ruggli and collaborators.

In our hands this reaction gave a good yield of an oil which is evidently the required cis-*stilbene*; it is easily isomerised by boiling it with nitrobenzene and a trace of iodine to the solid *trans*-form, m. p. 70—70·5°, which is also obtained by dehydrating the reaction product of 1-naphthaldehyde and benzylmagnesium chloride. This is evidently identical with the product prepared by Balla, whereas Thiele's hydrocarbon must have a different structure.

The corresponding β-naphthyl *compounds* were obtained by analogous procedures; here again the *cis*-form is an oil which was characterised as the picrate, whereas the trans-*form* obtained from it or by the Grignard reaction is a solid, m. p. 145—146°.

1-(1-Naphthyl)-2-(2-naphthyl)ethylene has been prepared by Badger (*J.*, 1941, 535) by the decarboxylation process and is evidently the *cis*-form because it is easily converted into a new *isomeride*, m. p. 191—192°. αβ-Di-(1-naphthyl)acrylic acid (Badger, *loc. cit.*) is decarboxylated to a new cis-*stilbene*, which is isomerised to the known compound, m. p. 164—165° (Elbs, *J. pr. Chem.*, 1893, **47**, 56) which must therefore be the *trans*-form. Similarly, the di-(2-naphthyl) acid gives a new *stilbene* which is converted into the known compound, m. p. 258° (Friedmann, *Ber.*, 1916, **49**, 277, gives m. p. 255°). The last two *trans*-stilbenes have more recently been prepared by Wood, Bacon, Meibohm, Throckmorton, and Turner (*J. Amer. Chem. Soc.*, 1941, **63**, 1334) by a method somewhat similar to that of Friedmann and of Elbs. We were unable to obtain either of them by the Grignard reaction.

Experimental.

(Melting points are uncorrected.)

1-*Phenyl*-2-(1-*naphthyl*)*ethylene*.—Benzylmagnesium chloride, prepared from 1 g. of magnesium and 4·8 g. of benzyl chloride in ether, was treated dropwise with an ethereal solution of 5·6 g. of 1-naphthaldehyde, the mixture being then warmed for 30 minutes. After decomposition with ammonium chloride the ethereal solution of the product was dried and evaporated, and the residue taken up in 100 c.c. of benzene and boiled for 1 hour with 10 g. of phosphoric oxide. The solution was then diluted with 100 c.c. of petroleum (b. p. 60—80°) and percolated through a column of activated alumina. The percolate on evaporation gave an oil which was converted into the picrate, m. p. 127—128° after crystallisation from benzene–petroleum. The hydrocarbon regenerated from it by passing its benzene solution through a column of alumina formed needles, m. p. 70—70·5°, from light petroleum (yield 1 g.) (Found : C, 93·5; H, 6·0. Calc. : C, 93·9; H, 6·1%).

α-Phenyl-β-(1-naphthyl)acrylic acid (Ruggli and Preuss, *loc. cit.*) (3 g.) was boiled with 30 c.c. of quinoline and 0·3 g. of copper chromate catalyst for 1 hour. The cooled mixture was treated with dilute hydrochloric acid and extracted with ether, and the extract, after being washed with water and then with alkali, was dried and evaporated. The residual oil was chromatographed in light petroleum solution and the recovered oil distilled; the main portion (1·5 g.) boiled at 170°/0·01 mm. and consisted of the cis-*stilbene* (Found : C, 93·7; 6·4. $C_{16}H_{14}$ requires C, 93·9; H, 6·1%). The oil was boiled for 15 minutes with 10 volumes of nitrobenzene and a crystal of iodine. The *trans*-compound recovered had m. p. and mixed m. p. 70° after crystallisation.

1-*Phenyl*-2-(2-*naphthyl*)*ethylene*.—A Grignard reagent prepared from 9·6 g. of benzyl chloride was treated with 11·8 g. of 2-naphthaldehyde exactly as described above and the product worked up in the same way. 4·5 G. of the *trans*-compound were obtained, needles from petroleum or alcohol, m. p. 145—146° (Found : C, 94·1; H, 6·4. $C_{16}H_{14}$ requires C, 93·9; H, 6·1%).

3 G. of 2-naphthaldehyde, 2·8 g. of sodium phenylacetate, 1 g. of anhydrous potassium carbonate, and 15 c.c. of acetic anhydride were boiled under reflux for 3 hours (oil-bath at 170°). *α-Phenyl-β-(2-naphthyl)acrylic acid* (1·9 g.) was isolated by extraction of the reaction product with sodium carbonate and acidification; it was crystallised from acetic acid, then from alcohol, and formed plates, m. p. 205—206° (Found : C, 83·2; H, 5·1. $C_{19}H_{14}O_2$ requires C, 83·0; H, 5·0%). The acid was decarboxylated and the product purified as described above. The earlier runnings from the chromatogram contained an oil; later fractions gave a solid on evaporation and this proved to be the *trans*-stilbene already described. The oil was converted into the picrate which melted at 68—69° in the crude state, but could not be purified as it dissociated on attempted crystallisation. The hydrocarbon regenerated from it remained oily, and the amount isolated did not permit complete purification; it must have consisted mainly of the *cis*-form because it was completely isomerised to the solid stilbene, m. p. 145—146°, on being heated with iodine in nitrobenzene solution.

1-(1-*Naphthyl*)-2-(2-*naphthyl*)*ethylene*.—The compound, m. p. 103—105° (Badger, *loc. cit.*), is readily isomerised to the trans-*isomeride* which crystallises from benzene–alcohol in plates, m. p. 191—192°; some degree of conversion takes place even on repeated crystallisation of the *cis*-form (Found : C, 94·4; H, 5·6. $C_{32}H_{14}$ requires C, 94·2; H, 5·8%).

1 : 2-*Di*-(1-*naphthyl*)*ethylene*.—*αβ*-Di-(1-naphthyl)acrylic acid was prepared as described by Badger; it was found to melt somewhat higher than the figure recorded by him, namely at 232—233°. The acid was accompanied by a small amount (about 4%) of a neutral by-product, which proved to be the known *trans*-form of the stilbene. After two purifications by chromatography in petroleum solution and two crystallisations from alcohol it melted at 164—165° (lit. 161°) (Found : C, 94·3; H, 5·8. Calc. : C, 94·2; H, 5·8%).

The acrylic acid (6 g.) was decarboxylated as described above giving 3·3 g. of the cis-*stilbene* which crystallised from alcohol in plates, m. p. 103—104° (Found : C, 93·9; H, 5·8. $C_{32}H_{14}$ requires C, 94·2;

H, 5·8%). The isomerisation was carried out as described above and afforded the pure *trans*-form, m. p. and mixed m. p. 164—165°.

1:2-*Di*-(2-*naphthyl*)*ethylene*.—In the preparation of 2-naphthaldehyde it was found convenient to prepare the intermediate bromomethylnaphthalene by boiling 2-methylnaphthalene (140 g.) with *N*-bromosuccinimide in carbon tetrachloride solution for 10 hours, using benzoyl peroxide as a catalyst (Schmid and Karrer, *Helv. Chim. Acta*, 1946, **29**, 573) (yield 120 g.). The aldehyde (8·5 g.) was condensed with potassium 2-naphthylacetate as described above; no neutral by-product appeared to be formed and the yield of *αβ-di*-(2-*naphthyl*)*acrylic acid* was 7·7 g. The acid was crystallised twice from acetic acid, then from alcohol and finally from benzene, m. p. 230° (Found: C, 85·2; H, 5·2. $C_{22}H_{16}O_2$ requires C, 85·2; H, 5·0%). Decarboxylation of 6·4 g. of the acid gave 3·5 g. of the cis-*stilbene*, plates from alcohol, m. p. 105·5—106·5° (Found: C, 94·5; H, 5·8. $C_{22}H_{16}$ requires C, 94·2; H, 5·8%). The *cis*-compound was completely isomerised on boiling with iodine in nitrobenzene to the *trans*-form, m. p. 258—259° after two crystallisations from benzene.

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323. *The Attempted Rearrangement of Some Arylbenziminoquinolyl Ethers.*

By D. MURIEL HALL.

Although N-*phenylbenzimino*-8-*quinolyl ether* undergoes normal rearrangement to 8-*benzanilidoquinoline*, introduction of an *ortho*-halogen atom into the aryl group causes the ether to decompose at temperatures at which rearrangement might be expected to occur. A compound with halogen atoms in the quinoline nucleus underwent normal rearrangement.

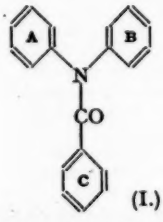
JAMISON and TURNER (*J.*, 1937, 1954) applied Chapman's diphenylamine synthesis to the preparation of substituted *N*-benzoyldiphenylamine-2-carboxylic acids, the salts of which, with optically stable bases, provided suitable material for the study of asymmetric transformation (*idem*, *J.*, 1938, 1646; 1940, 264). No study of the equilibria obtaining in the case of salts of optically labile *bases* with optically stable acids has yet been made, and in pursuit of this object the preparation of *N*-benzoyldiphenylamines with basic properties has now been attempted. It did not, however, prove possible to prepare compounds with basic substituents in either ring A or ring C (I), though a limited number of compounds containing a substituted quinoline nucleus in place of ring B were obtained.

The action of phosphorus pentachloride on benz-*m*-dimethylaminoanilide, 8-benzamidoquinoline, and N-*benzoyl*-N′-*acetyl*-N′-*methyl*-p-*phenylenediamine* in each case failed to give the desired iminochloride (or iminochloride hydrochloride).

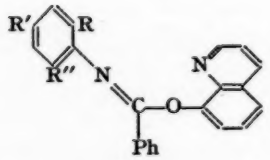
p-*Dimethylaminobenz*-o-*toluidide* was obtained in very poor yield by an adaptation of Human and Mills's method (*Nature*, 1946, **158**, 877).

Although 2-*bromo*-2′-*nitro*-N-*benzoyl*-4-*methyldiphenylamine* was prepared, its reduction to the 2′-amino-compound was unsatisfactory.

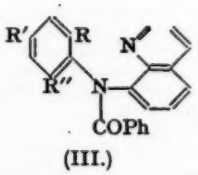
Phenylbenziminochloride reacted readily with the sodium derivative of 8-hydroxyquinoline to give N-*phenylbenzimino*-8-*quinolyl ether* (II; R = R′ = R″ = H) which rearranged at about 280° to 8-*benzanilidoquinoline* (III; R = R′ = R″ = H). The rearrangement was also



(I.)



(II.)



(III.)

successful when R = Me, R′ = R″ = H; but when R was a halogen atom (*e.g.*, R = Cl, R′ = R″ = H) the ether decomposed on heating at about 270°, while at lower temperatures some unchanged ether was recovered from the tarry product. The compounds (II; R = Br, R′ = Me, R″ = H) and (II; R = Br, R′ = R″ = Me) also decomposed when rearrangement

was attempted and thus behaved similarly to certain nitro-substituted imino-ethers investigated by Chapman (*J.*, 1927, 1743).

In the one compound investigated, the introduction of a halogen atom into the quinoline nucleus did not affect the rearrangement and N-*phenylbenzimino*-5 : 7-*dichloro*-8-*quinolyl ether* passed smoothly into 5 : 7-*dichloro*-8-*benzanilidoquinoline* at 260°, but, as the ether was difficult to prepare and the product was only sparingly soluble, the use of 5 : 7-dichloro-8-hydroxyquinoline was not further explored.

Experimental.

Microanalyses are by Drs. Weiler and Strauss, Oxford; m. p.s are uncorrected.

Benz-m-*dimethylaminoanilide*.—*m*-Nitroaniline was methylated by Ullmann's method (*Annalen*, 1903, **327**, 104), reduced with stannous chloride and hydrochloric acid, and the crude *NN*-dimethyl-*m*-phenylenediamine treated with benzoyl chloride in the presence of 10% aqueous sodium hydroxide. The resulting benzoyl compound (20 g.) was heated on the water-bath with phosphorus pentachloride (17·5 g.) until reaction ceased (3 hours); on being washed with light petroleum it set to a hard, deliquescent orange solid. This was filtered off quickly and added to an alcoholic solution of the sodium derivative of methyl salicylate in the presence of excess of sodium ethoxide. Normal working up gave a gum from which some crystals of the original benz-*m*-dimethylaminoanilide separated several weeks later. No other solid product could be isolated.

8-*Benzamidoquinoline*.—Quinoline was nitrated (Meigen, *J. pr. Chem.*, 1908, **77**, 472) and the resulting mixture of 5- and 8-nitroquinolines separated through the nitrates (Dufton, *J.*, 1892, **61**, 782). Reduction of 8-nitroquinoline by iron and 50% acetic acid (Dikshoorn, *Rec. Trav. chim.*, 1929, **48**, 147) gave a poor yield, but 8-aminoquinoline was obtained almost quantitatively by employing iron and water in presence of a little acetic acid. Schotten–Baumann benzoylation gave 8-benzamidoquinoline in 90% yield. This was heated with a slight excess of phosphorus pentachloride, alone, or with phosphoryl chloride as solvent, and the resulting purple solid washed with light petroleum and added to an alcoholic solution of the sodium derivative of methyl salicylate. No crystalline product could be isolated.

N-*Benzoyl*-N′-*acetyl*-N′-*methyl*-p-*phenylenediamine*.—*N*-Methylacetanilide was prepared rapidly and in good yield by adding acetic anhydride (56 g.) to a mixture of methylaniline (54 g.), water (200 g.), and ice (100 g.). The whole was cooled in ice-water and stirred vigorously. After a few minutes the emulsion cleared and almost at once set to a thick paste. The acetyl compound was filtered off and a further crop obtained by adding a paste of sodium hydrogen carbonate (25 g.) to the filtrate; total yield 66 g., 88%; m. p. 100—101°. This was nitrated (Morgan and Grist, *J.*, 1918, **113**, 688), and reduced with iron, water, and acetic acid to *p*-amino-*N*-methylacetanilide (yield 80—90% after one crystallisation from water). Benzoylation in pyridine solution gave N-*benzoyl*-N′-*acetyl*-N′-*methyl*-p-*phenylenediamine*, which crystallised from alcohol in long prisms, m. p. 203—204° (Found: C, 71·7; H, 5·7; N, 10·6. $C_{16}H_{16}O_2N_2$ requires C, 71·6; H, 6·0; N, 10·4%) (yield 66%). Phosphorus pentachloride reacted with the above compound to give a dark sticky mass which began to decompose during the attempt to remove phosphoryl chloride by distillation. It was washed with light petroleum and became hard but not crystalline. It showed none of the usual reactions of an iminochloride or iminochloride hydrochloride and was not investigated further.

p-*Dimethylaminobenz*-o-*toluidide*.—(*a*) *p*-Dimethylaminobenzoic acid (Willstätter and Kahn, *Ber.*, 1904, **37**, 401) was treated with thionyl chloride, and *o*-toluidine added to the product. None of the required compound could be isolated. (*b*) The acid chloride was prepared from dimethylaniline (48·5 g.) and carbonyl chloride (10 g.) (Rivier and Richard, *Helv. Chim. Acta*, 1925, **8**, 490), and *o*-toluidine (11 g.) added. 9 G. of the toluidide, contaminated with di-*o*-tolylurea, were obtained, but crystallisation from a variety of solvents failed to give a sharply melting specimen. (*c*) (Method of Human and Mills, *loc. cit.*). Thionyl chloride (24 g.) was added gradually to *p*-dimethylaminobenzoic acid (33 g.) dissolved in pyridine (165 c.c.). After this had been stirred for 1 hour, a solution of *o*-toluidine (21 g.) in pyridine (16 c.c.) was added, and the mixture left overnight. It was poured into water, and the solid separated and dissolved in dilute hydrochloric acid. The insoluble di-*o*-tolylurea was filtered off, and the toluidide precipitated by making the filtrate just alkaline with sodium hydroxide solution. The crude product was extracted with boiling benzene, and the extract dried and concentrated. It deposited 11 g. (22%) of the *toluidide*, m. p. 138—140°. Two further crystallisations from benzene gave diamond-shaped, flattened prisms, m. p. 141—142° (Found: C, 75·3; H, 7·1; N, 10·4. $C_{16}H_{18}ON_2$ requires C, 75·6; H, 7·1; N, 11·0%). The *picrate* crystallised from alcohol in long, irregular, yellow prisms, m. p. 155° (Found: C, 55·5; H, 4·3. $C_{16}H_{18}ON_2,C_6H_3O_7N_3$ requires C, 54·7; H, 4·4%).

N-o-*Bromo*-p-*tolylbenzimino*-o-*nitrophenyl ether* was obtained in 67% yield from benz-*o*-bromo-*p*-toluidideiminochloride and *o*-nitrophenol in presence of alcoholic sodium ethoxide. It crystallised from alcohol in six-sided yellow plates, m. p. 130—131° (Found: Br, 19·7. $C_{26}H_{18}O_3N_2Br$ requires Br, 19·4%).

2-*Bromo*-2′-*nitro*-N-*benzoyl*-4-*methyldiphenylamine*.—The above ether rearranged on heating for 1 hour in a bath kept at 170—180°, and the *amine* crystallised from ethyl acetate in pale yellow rhombs, m. p. 152—153° (Found: C, 58·5; H, 4·5; Br, 20·15. $C_{20}H_{15}O_3N_2Br$ requires C, 58·4; H, 3·7; Br, 19·4%).

N-*Phenylbenzimino*-8-*quinolyl Ether* (II; R = R′ = R″ = H).—8-Hydroxyquinoline (63 g.), dissolved in alcohol (500 c.c.) at 35°, and benzanilideiminochloride (94 g.), dissolved in dry ether (300 c.c.), were added in quick succession to a cooled solution of sodium (10 g.) in alcohol (500 c.c.). Sodium chloride separated at once. After 12 hours the ether and most of the alcohol were distilled off, and the residue poured into water. The solid was collected and crystallised from a large volume of alcohol; yield 105 g., 74%. N-*Phenylbenzimino*-8-*quinolyl ether* was obtained as almost colourless, irregular, stout prisms, m. p. 154—155° (Found: C, 80·9; H, 5·0; N, 8·8. $C_{22}H_{16}ON_2$ requires C, 81·45; H, 5·0; N, 8·6%). This compound, like several other iminoquinolyl ethers, was a nose irritant.

8-*Benzanilidoquinoline* (III; R = R′ = R″ = H).—The above ether (20 g.) was heated for 20 minutes

in a wide tube in a metal-bath kept at 280°. The reaction was strongly exothermic. Crystallisation from alcohol gave 14·5 g. (72%) of a brownish product, m. p. 129—131°. It was purified by recrystallisation from benzene and then from alcohol, 8-*benzanilidoquinoline* being obtained as colourless sheaves of needles, m. p. 130—131° (Found: C, 81·1; H, 5·0; N, 9·25. $C_{22}H_{16}ON_2$ requires C, 81·45; H, 5·0; N, 8·6%).

N-o-*Tolylbenzimino*-8-*quinolyl Ether* (II; R = Me, R′ = R″ = H).—This *ether* was prepared (47% yield) as described above by treating the sodium derivative of 8-hydroxyquinoline with benz-*o*-toluididеiminochloride. It crystallised from alcohol in rectangular plates, m. p. 115—116° (Found: C, 81·1; H, 5·3; N, 8·1. $C_{23}H_{18}ON_2$ requires C, 81·6; H, 5·4; N, 8·3%). On heating for 10 minutes in a bath at 280° it rearranged to give 8-*benz*-o-*toluididoquinoline* (III; R = Me, R′ = R″ = H), which crystallised from alcohol in rhombohedra, m. p. 127—128° (Found: C, 81·3; H, 5·5; N, 8·5. $C_{23}H_{18}ON_2$ requires C, 81·6; H, 5·4; N, 8·3%).

N-o-*Chlorophenylbenzimino*-8-*quinolyl ether* (II; R = Cl, R′ = R″ = H) was obtained from benz-*o*-chloroanilideiminochloride and 8-hydroxyquinoline in 71% yield. It crystallised from acetone in pale yellow prisms, m. p. 133° (Found: C, 73·6; H, 4·0; N, 7·1; Cl, 9·3. $C_{22}H_{15}ON_2Cl$ requires C, 73·6; H, 4·2; N, 7·8; Cl, 9·9%). When heated in a bath kept at 280° or at 240° it decomposed.

N-o-*Bromo*-p-*tolylbenzimino*-8-*quinolyl ether* (II; R = Br, R′ = Me, R″ = H) was obtained in 62% yield from benz-*o*-bromo-*p*-toluididеiminochloride and 8-hydroxyquinoline. It was crystallised from alcohol and then from carbon tetrachloride and obtained as diamond-shaped prisms, m. p. 135·5° (Found: C, 66·3; H, 4·2; N, 6·6; Br, 19·5. $C_{23}H_{17}ON_2Br$ requires C, 66·2; H, 4·1; N, 6·7; Br, 19·15%). Rearrangement was attempted at 270—280° (vigorous decomposition) and at 240° and 220°, but without success.

N-5-*Bromo*-4-m-*xylylbenzimino*-8-*quinolyl Ether* (II; R = Br, R′ = R″ = Me).—Benz-5-bromo-4-*m*-xylidide was converted into benz-5-bromo-4-*m*-xylididеiminochloride, b. p. 248°/31 mm. (74% yield), which with 8-hydroxyquinoline gave a 66% yield of the *ether*, crystallising from alcohol in rectangular prisms, m. p. 116—117° (Found: C, 66·1; H, 4·5; N, 6·9; Br, 18·6. $C_{24}H_{19}ON_2Br$ requires C, 66·8; H, 4·4; N, 6·5; Br, 18·5%). It decomposed on heating at 260°.

N-*Phenylbenzimino*-5 : 7-*dichloro*-8-*quinolyl Ether*.—5 : 7-Dichloro-8-hydroxyquinoline (36 g.) was partly dissolved in benzene (450 c.c.) and added to sodium ethoxide solution (4 g. of sodium in 150 c.c. alcohol); immediate precipitation of a mixture of 5 : 7-dichloro-8-hydroxyquinoline and its sodium derivative occurred. Benzanilideiminochloride (36 g.), dissolved in benzene (100 c.c.), was added, and the mixture left with occasional shaking for 40 hours, during which most of the solid disappeared. The remaining solid (18 g.) was filtered off: concentration of the filtrate gave 26 g. of mixed product. Further crops of 7 g., 3 g., and 3 g. severally were obtained by dilution with light petroleum. Lengthy fractional crystallisations from benzene and alcohol effected separation of the product into 5 : 7-dichloro-8-hydroxyquinoline (m. p. and mixed m. p. with authentic specimen, 182—183°) and N-*phenylbenzimino*-5 : 7-*dichloro*-8-*quinolyl ether* (14 g.; 21%), which crystallised from alcohol in rods, m. p. 163° (Found: C, 67·0; H, 3·5; N, 7·0; Cl, 18·3. $C_{22}H_{14}ON_2Cl_2$ requires C, 67·2; H, 3·6; N, 7·1; Cl, 18·0%).

5 : 7-*Dichloro*-8-*benzanilidoquinoline* was obtained by heating the above ether for a few minutes at 260° (bath temp.). It crystallised from benzene in rhombs, m. p. 214—215° (Found: C, 67·4; H, 3·9; N, 7·2; Cl, 17·7. $C_{22}H_{14}ON_2Cl_2$ requires C, 67·2; H, 3·6; N, 7·1; Cl, 18·0%). It was sparingly soluble in alcohol, acetone, ethyl acetate, and benzene.

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UNIVERSITY OF LONDON (BEDFORD COLLEGE). [*Received, November 19th*, 1947.]

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324. *The Chemistry of Bacteria. Part I. The Synthesis of Hydroxyindoles.*

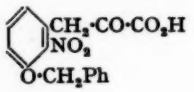
By R. J. S. BEER, KENNETH CLARKE, H. G. KHORANA, and ALEXANDER ROBERTSON.

The synthesis of 6- and 7-hydroxyindole by way of the benzyl ethers of the requisite *o*-nitrohydroxyphenylpyruvic acid is described. By application of Nenitzescu's reaction (*Ber.*, 1925, **58**, 1063) the synthesis of 4-hydroxyindole and of 5-hydroxy-2-methylindole together with a new synthesis of 5-hydroxyindole has been achieved from the corresponding 2 : β-dinitroacetoxystyrenes.

IN the course of degradation experiments on the pigment, violacein, from *Chromobacterium violaceum* it became necessary to synthesise indoles hydroxylated in the benzene nucleus as reference compounds in attempts to identify two isomeric products, C_8H_7ON, obtained in small amounts, one of which gave a strong reaction with Ehrlich's reagent. Apart from studies on 5:6-dihydroxyindole and its derivatives in connection with melanin production and related topics (*e.g.*, Raper, *Biochem. J.*, 1927, **21**, 89; 1930, **24**, 239; Burton, *J.*, 1932, 546; Bergel and Morrison, *J.*, 1943, 48), comparatively little work has been recorded on the synthesis of the required type of hydroxyindole. Blaikie and Perkin (*J.*, 1924, **125**, 296) achieved the synthesis of 4-, 5-, and 7-methoxyindole by a general method, but were unable to demethylate their products, although Hoshino and Shimodaira (*Annalen*, 1935, **520**, 19) record the de-ethylation of bufotenine ethyl ether. Similarly, Burton and Stoves (*J.*, 1937, 1726) prepared 5- and 6-benzyloxyindole

by the Blaikie–Perkin route but did not succeed in removing the benzyl residues, whereas Bergel and Morrison (*J.*, 1943, 49) without reference to the original describe the preparation of Burton and Stoves's 5-benzyloxyindole-2-carboxylic acid which they succeeded in debenzylating by hydrogenolysis and in subsequently converting into 5-hydroxyindole. The use of the carboxyl groups in the α-position to inhibit the hydrogenation of the heterocyclic system finds a close parallel in the work of Foster and Robertson (*J.*, 1939, 921, 930) on the analogous coumarone-2-carboxylic acids.

In the present studies 6-*hydroxyindole* has been prepared by hydrogenolysis of 6-benzyloxyindole-2-carboxylic acid (Burton and Stoves, *loc. cit.*) followed by decarboxylation of the resulting 6-*hydroxyindole-2-carboxylic acid*.



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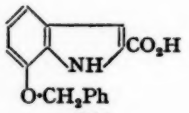

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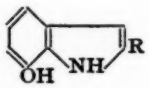
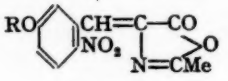


(III.)

For the synthesis of 7-*hydroxyindole* (III; R = H) by the same route, the condensation of 2-*nitro*-3-*benzyloxytoluene* with ethyl oxalate followed by hydrolysis of the product gave the pyruvic acid (I) which was characterised by the formation of the *oxime*, and was subsequently converted into (III; R = H) by way of the stages (II) and (III; R = CO_2H). Unlike the behaviour of 6-hydroxyindole-2-carboxylic acid which gave a 53% yield of the indole, the yield of (III; R = H) from (III; R = CO_2H) was extremely small. Attempts to synthesise 4-*hydroxyindole* from 2-*nitro*-6-*benzyloxytoluene* by this route were abandoned because when the latter reacted with ethyl oxalate in the presence of potassium ethoxide only small amounts of resinous material were obtained which had the properties of a pyruvic acid but which could not be purified economically. On being submitted to reduction and cyclisation the crude acid gave a product from which only a trace of a crystalline substance exhibiting an Ehrlich reaction was isolated.



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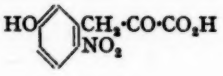

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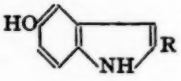

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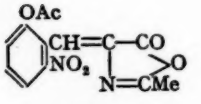

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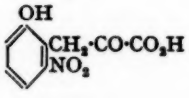

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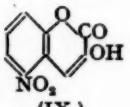
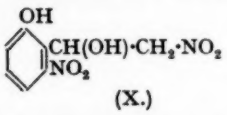


(IX.)

Where the requisite *o*-nitrobenzaldehyde is available, the azlactone method affords a convenient route to the required hydroxy-*o*-nitrophenylpyruvic acids for the synthesis of hydroxyindoles, and accordingly a new synthesis of 5-hydroxyindole-2-carboxylic acid (VI; R = CO_2H) was developed, the 5-keto-2-methyl-4 : 5-dihydro-oxazole from aceturic acid being used in place of the corresponding phenyl derivative because of the difficulties encountered in the hydrolysis of 5-keto-2-phenyl-4-(2′-nitrobenzylidene)-4 : 5-dihydro-oxazoles (cf. Burton, *J.*, 1935, 1265). Condensation of 2-nitro-5-hydroxybenzaldehyde with aceturic acid by means of sodium acetate and acetic anhydride gave the *azlactone* (IV; R = H) and its *acetate* (IV; R = Ac), either of which on hydrolysis with hot hydrochloric acid furnished the *pyruvic acid* (V) in good yield. Reduction of (V) and simultaneous cyclisation of the resulting amino-compound afforded 5-hydroxyindole-2-carboxylic acid, identical with a specimen obtained from its benzyl ether (*loc. cit.*). In a projected synthesis of 4-hydroxyindole from 2-nitro-6-hydroxybenzaldehyde by the same route, the *azlactone* (VII) was obtained in satisfactory yield, and, as would be expected, the pyruvic acid (VIII) formed by the acid hydrolysis of (VII) immediately cyclised to form 5-*nitro*-3-*hydroxycoumarin* (IX). We were unable, however, to discover the requisite conditions for the reduction of the pyruvic acid (VIII) regenerated from (IX) combined with the cyclisation of the resulting amino-acid to yield the 4-hydroxyindole-2-carboxylic acid.

In view of the failure to obtain 4-hydroxyindole by the foregoing methods, we investigated

the application of Nenitzescu's apparently little-known procedure for converting 2 : β-dinitrostyrene into indole (*Ber.*, 1925, **58**, 1063) to the synthesis of hydroxyindoles.



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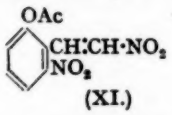

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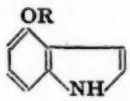
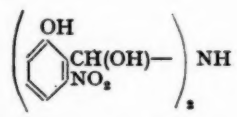
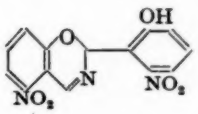


(XII.)

2-Nitro-6-hydroxybenzaldehyde was condensed with nitromethane by means of alcoholic potassium hydroxide, and the resulting crude nitro-alcohol (X) simultaneously dehydrated and acetylated with the formation of 2 : β-*dinitro*-6-*acetoxystyrene* (XI). Reduction of (XI) with iron filings and acetic acid gave rise to 4-*acetoxyindole* (XII; R = Ac) which on deacetylation by the methyl-alcoholic ammonia method furnished 4-*hydroxyindole* (XII; R = H). By an analogous series of reactions 5-hydroxyindole was prepared from 2-nitro-5-hydroxybenzaldehyde by way of 2 : β-*dinitro*-5-*acetoxystyrene* and 5-*acetoxyindole*, a procedure which avoids the wasteful decarboxylation process; the overall yield from the aldehyde was 35—40%. When nitromethane was replaced by nitroethane in the condensation with 2-nitro-5-hydroxybenzaldehyde, a somewhat lower yield of the homologous 2 : β-*dinitro*-5-*acetoxy*-β-*methylstyrene* was obtained which was successively converted into 5-*acetoxy*- and 5-*hydroxy*-2-*methylindole* by the standard method.

(XIII.)





(XIV.)

For condensing aromatic aldehydes with nitromethane, Rao and co-workers (*Helv. Chim. Acta*, 1929, **12**, 58) recommend the use of ammonium acetate in acetic acid, but when this reagent was applied to 2-nitro-6-hydroxybenzaldehyde the nitro-compound (X) was not formed. Instead, a good yield of a well-crystallised product, $C_{14}H_9O_6N_3$, was obtained which was phenolic and gave a *monoacetate*. Further, it was subsequently discovered that the nitromethane did not take part in the reaction, and therefore it seems reasonably certain that the substance is the *benz*-1 : 3-*oxazine* (XIV), probably formed by way of the intermediate benzaldehyde-ammonia compound (XIII).

Further experimental work on (XIV) and its homologues is in hand.

Experimental.

2-*Nitro*-5-*hydroxyphenylpyruvic Acid* (V).—An intimate mixture of 2-nitro-5-hydroxybenzaldehyde (5 g.), aceturic acid (6 g.), sodium acetate (6 g.), and acetic anhydride (15 ml.) was heated on the steam-bath for 1½ hours, kept for 10 days, and treated (agitate) with methanol (10 ml.) followed by water (5 ml.). 2 Hours later the solid (4·8—5·6 g.) was collected and dissolved in warm alcohol. On cooling, the solution deposited 5-*keto*-2-*methyl*-4-(2′-*nitro*-5′-*acetoxybenzylidene*)-4 : 5-*dihydro-oxazole* (IV; R = Ac) in yellow needles, m. p. 140°, after recrystallisation from the same solvent (Found : C, 53·9; H, 3·7; N, 9·8. $C_{13}H_{10}O_6N_2$ requires C, 53·8; H, 3·5; N, 9·7%). Concentration of the first alcoholic liquor and then dilution with water gave a pale yellow solid which on crystallisation from aqueous alcohol gave the *hydroxybenzylidene*-4 : 5-*dihydro-oxazole* (IV; R = H) in colourless needles, m. p. 173° (Found : C, 53·4; H, 3·4; N, 11·2. $C_{11}H_8O_5N_2$ requires C, 53·2; H, 3·2; N, 11·3%).

Concentrated sulphuric acid (20 ml.) was gradually added to water (25 ml.) containing a suspension of the foregoing mixed azlactones (4 g.), and the resulting solution boiled for 1 minute. On cooling, 2-*nitro*-5-*hydroxyphenylpyruvic acid* separated; it crystallised from concentrated hydrochloric acid–water (1 : 1) in colourless prisms (3·2 g.), m. p. 194° (decomp.), having a green ferric reaction in alcohol (Found : C, 48·1; H, 3·3; *M*, by titration, 223·6. $C_9H_7O_6N$ requires C, 48·0; H, 3·1%. *M*, 225). The same acid (1·35 g.), m. p. 194° (decomp.), was obtained when the azlactone mixture (2 g.) was boiled with 1·5N-hydrochloric acid (35 ml.) for 4—5 hours and the product isolated with ether. Oximation of the acid with hydroxylamine and aqueous sodium hydroxide for ½ hour on the steam-bath gave the *oxime* which formed colourless needles, m. p. 181°, from aqueous alcohol (Found : C, 45·2; H, 3·5; N, 11·8. $C_9H_8O_6N_2$ requires C, 45·0; H, 3·3; N, 11·7%).

5-*Hydroxyindole* (VI; R = H).—Ferrous sulphate (44 g.) dissolved in water (48 ml.) was added to a solution of the foregoing pyruvic acid (5 g.) in aqueous ammonia (34 ml.; *d* 0·88) and water (14 ml.), and the mixture gently refluxed for 1½ hours. The mixture was filtered, the solid extracted several times with warm dilute aqueous ammonia (500 ml.), and the combined filtrates acidified and repeatedly extracted with ether. Evaporation of the dried ethereal extracts left 5-hydroxyindole-2-carboxylic acid (3 g.), m. p. 246° (decomp.), after purification from water, identical with a specimen prepared by route employed by Bergel and Morrison (*loc. cit.*). When a solution of the acid from either source (0·5 g.) in glycerol (5 ml.) was kept at 225—230°, carbon dioxide was evolved, and after 25 minutes the solution was cooled, diluted with water, and extracted several times with ether. The red residue left on the evaporation of the ether was extracted with light petroleum (b. p. 80—100°); on concentration the solution deposited

5-hydroxyindole, m. p. 107°, after sublimation and recrystallisation, identical with material prepared by the method of Bergel and Morrison (*loc. cit.*); yield, 0·25 g. of indole and 0·8 g. of unchanged acid from 2·5 g. of acid (Found: C, 72·3; H, 5·4. Calc. for C_8H_7ON: C, 72·2; H, 5·3%). Addition of a hot benzene solution of the hydroxyindole to picric acid in the same solvent gave a red precipitate of the *picrate* which separated from benzene–light petroleum (b. p. 60—80°) in elongated orange-red needles, m. p. 167° (Found: N, 15·3. $C_8H_7ON,C_6H_3O_7N_3$ requires N, 15·5%).

In view of the poor yields obtained in the decarboxylation process, a number of modifications of the method were investigated with unsuccessful results; *e.g.*, heating 5-hydroxyindole-2-carboxylic acid (*a*) in diphenyl ether at 210° for 10 minutes and then at 250° for 1 minute gave unchanged material, (*b*) with aniline at 184° gave a dark resin, (*c*) with boiling quinoline containing copper-bronze gave tar and a little unchanged acid, (*d*) at 250° in a vacuum gave a sublimate of acid.

6-*Hydroxyindole*.—2-Nitro-4-benzyloxyphenylpyruvic acid (18·4 g.) was prepared by condensation of 2-nitro-4-benzyloxytoluene (Burton and Stoves, *loc. cit.*) (24 g.) and ethyl oxalate (15 g.) in ether (350 ml.) with potassium ethoxide (from 4 g. of potassium) in the course of 18 hours, and hydrolysis of the resulting ester with warm 5% aqueous sodium hydroxide (250 ml.) during 1 hour. The aqueous layer was separated, washed twice with a little ether, and acidified with hydrochloric acid, yielding the pyruvic acid as an oil which solidified and then separated from dilute acetic acid in yellow plates (18·4 g.), m. p. 94° (Found: C, 60·9; H, 4·3; N, 4·3. Calc. for $C_{16}H_{13}O_6N$: C, 61·0; H, 4·1; N, 4·4%) (cf. Burton and Stoves, who give m. p. 89—90° for this acid and state that it forms a hydrate, for which they record analysis). The oxime separated from aqueous alcohol in colourless needles, m. p. 182° (decomp.) (Found: N, 8·4. $C_{16}H_{14}O_6N_2$ requires N, 8·5%). Simultaneous reduction and cyclisation of this acid gave 6-benzyloxyindole-2-carboxylic acid, m. p. 200° (decomp.) (Burton and Stoves give m. p. 185—186°) (Found: C, 71·8; H, 4·9; N, 5·2. Calc. for $C_{16}H_{13}O_3N$: C, 71·9; H, 4·9; N, 5·2%). Debenzylation of this benzyl ether (2 g.) with hydrogen and a 10% palladium–charcoal catalyst (2 g.) in acetic acid (100 ml.) was complete in 10 minutes, and ½ hour later the product was isolated by evaporation of the filtered solution in a vacuum and crystallised from water (charcoal), giving 6-*hydroxyindole-2-carboxylic acid* in glistening needles (0·9 g.), m. p. 236° (decomp.), which gave a red colouration with Ehrlich's reagent (Found: C, 61·1; H, 4·1; N, 8·0. $C_9H_7O_3N$ requires C, 61·0; H, 4·0; N, 7·9%). Methanol can be used as a solvent in place of acetic acid in the debenzylation process.

Decarboxylation of the indolecarboxylic acid (0·5 g.) in glycerol at 220—230° during 20 minutes gave 6-*hydroxyindole* which was purified by crystallisation from light petroleum (b. p. 80—100°), followed by sublimation in a vacuum, and then recrystallisation, being ultimately obtained in colourless needles (0·2 g.), m. p. 125·5° (Found: C, 72·3; H, 5·4; N, 10·8. C_8H_7ON requires C, 72·2; H, 5·3; N, 10·5%). The *picrate* formed dark red needles, m. p. 154—156° (decomp.), from benzene (Found: N, 15·3%).

2-*Nitro-3-benzyloxyphenylpyruvic Acid* (I).—2-Nitro-3-hydroxytoluene (Gibson, *J.*, 1923, 1269) (5 g.) was benzylated with benzyl bromide (3·8 ml.) and excess of potassium carbonate in boiling acetone (100 ml.) during 2½ hours; on isolation the *ether* was distilled in a high vacuum and then crystallised from light petroleum (b. p. 40—60°), forming colourless needles (6·5 g.), m. p. 36° (Found: N, 5·6. $C_{14}H_{13}O_3N$ requires N, 5·8%). This compound (24 g.) was condensed with ethyl oxalate (15 g.) by means of potassium ethoxide (from 4 g. of potassium) by the method used for 2-nitro-4-benzyloxytoluene; the resulting pyruvic acid was obtained in yellow plates (15·5 g.), m. p. 125°, which gave an *oxime* forming colourless needles, m. p. 190—191°, from aqueous alcohol (Found: C, 58·3; H, 4·4; N, 8·6. $C_{16}H_{14}O_6N_2$ requires C, 58·2; H, 4·2; N, 8·5%).

7-*Hydroxyindole* (III; R = H).—Simultaneous reduction and cyclisation of the foregoing nitropyruvic acid (3·8 g.) gave 7-*benzyloxyindole-2-carboxylic acid* (II) which crystallised from dilute acetic acid in needles (2·2 g.), m. p. 164° (Found: C, 71·8; H, 5·0; N, 5·0. $C_{16}H_{13}O_3N$ requires C, 71·9; H, 4·9; N, 5·2%). Debenzylation of this ether (2 g.) by the hydrogenation method furnished 7-*hydroxyindole-2-carboxylic acid* (III; R = CO_2H) which formed needles (1 g.), m. p. 252°, from water, almost insoluble in light petroleum or benzene and readily soluble in alcohol or acetone (Found: C, 61·2; H, 4·1; N, 8·0%). Decarboxylation of this acid (0·5 g.) by the glycerol process gave 7-*hydroxyindole* which was purified by sublimation in a vacuum and recrystallisation from light petroleum (b. p. 40—60°), forming colourless prisms (20 mg.), m. p. 96°, moderately soluble in benzene or chloroform and readily soluble in alcohol or ethyl acetate (Found: C, 72·4; H, 5·5; N, 10·8%).

2 : β-*Dinitro-6-acetoxystyrene* (XI).—Potassium hydroxide (0·7 g.) dissolved in alcohol (10 ml.) cooled to − 10° was added in the course of 20 minutes to an agitated solution of 2-nitro-6-hydroxybenzaldehyde (1 g.) and nitromethane (0·4 g.) in alcohol kept at − 10°. The mixture was then carefully acidified with cooled hydrochloric acid, and after dilution with water the condensation product, which usually remained in solution, was isolated with ether, being obtained as a yellow oil which slowly solidified. When this material was gently warmed with sodium acetate (2 g.) and acetic anhydride (3 ml.) for 15 minutes, and water (20 ml.) subsequently added, 2 : β-*dinitro-6-acetoxystyrene* separated as a gum which on trituration with water formed a granular crystalline powder (1·35 g.), m. p. 128—130°. Crystallised from alcohol, the acetate formed light tan plates having the same m. p. (Found: C, 47·5; H, 3·0; N, 11·3. $C_{10}H_8O_6N_2$ requires C, 47·6; H, 3·2; N, 11·1%).

4-*Hydroxyindole* (XII; R = H).—The reaction initiated by gently warming a mixture of 2-β-dinitro-6-acetoxystyrene (0·5 g.), iron filings (2 g.), alcohol (4 ml.), and acetic acid (4 ml.) until there was a vigorous evolution of hydrogen was allowed to continue for 10—12 minutes (a longer reaction period decreased the yield) with occasional heating. After filtration to remove insoluble material (wash several times with a little warm alcohol) the filtrate was diluted with water, basified with sodium hydrogen carbonate, and repeatedly extracted with ether. Evaporation of the combined, dried extracts left a pale greenish gum from which 4-*acetoxyindole* (0·1—0·12 g.) was isolated by extraction with excess of warm light petroleum (b. p. 60—80°), and crystallised from the same solvent, forming glistening needles, m. p. 100° (Found: C, 69·1; H, 5·4; N, 8·2. $C_{10}H_9O_2N$ requires C, 68·6; H, 5·1; N, 8·0%).

A solution of this acetate (0·1 g.) in methanol (3 ml.) was saturated at 0° with ammonia and then kept at below 5° for 16 hours. After the removal of the ammonia and the solvent in a vacuum followed by the addition of water (50 ml.), the product was isolated with ether. Extraction of this material with several

portions of boiling light petroleum (b. p. 80—100°) and concentration of the combined extracts gave *4-hydroxyindole* in elongated needles (yield, 70—80%), m. p .98°, unchanged on recrystallisation (Found : C, 72·0; H, 5·1; N, 10·3%). A mixture of this substance and its acetate had m. p. 75°. The *picrate* separated from benzene in red needles, which on being heated became partly yellow at 150° and then charred at 180° (Found : N, 15·2%). This indole, which is readily soluble in the usual organic solvents except light petroleum, gives a dark blue colour with alcoholic ferric chloride, and red solution exhibiting a strong green fluorescence with Ehrlich's reagent in the cold. On being warmed, the latter solution acquires a bluish tint, becomes turbid, and then deposits a green precipitate. On being kept a solution of the substance in tap water becomes blue.

2 : *β-Dinitro-5-acetoxystyrene* was prepared in almost theoretical yield from 2-nitro-5-hydroxybenzaldehyde by the method employed for the isomeric 6-acetoxy-derivative. Crystallised from alcohol, the substance formed straw-coloured needles, m. p. 118—119° (Found : C, 47·9; H, 3·2; N, 10·9%). Reduction of this nitrostyrene furnished *5-acetoxyindole* which separated from light petroleum (b. p. 60—80°) in a mass of lustrous micaceous plates, m. p. 113—114°; yield, 50—55% (Found : C, 68·8; H, 5·0; N, 7·7%). Deacetylation of this derivative by the alcoholic-ammonia process gave 5-hydroxyindole, identical with an authentic sample.

2 : *β-Dinitro-5-acetoxy-β-methylstyrene*.—2-Nitro-5-hydroxybenzaldehyde (1 g.) was condensed with nitroethane (0·5 g.) by the procedure employed for nitromethane, but the product from the acetylation process was less pure and in some experiments did not solidify completely. The crude *acetoxystyrene* (0·8—0·9 g.) was triturated with a little alcohol, and the resulting granular product (0·7 g.) was crystallised from the same solvent, forming pale brownish hexagonal plates, m. p. 88—89° (Found : C, 49·4; H, 4·0; N, 10·2. $C_{11}H_{10}O_6N_2$ requires C, 49·6; H, 3·8; N, 10·5%).

5-Hydroxy-2-methylindole.—Reduction of the foregoing styrene (0·5 g.) with iron filings and acetic acid furnished *5-acetoxy-2-methylindole* which separated from light petroleum (b. p. 80—100°) in colourless needles (0·09—0·1 g.), m. p. 128—130° (Found : C, 69·6; H, 5·9; N, 7·8. $C_{11}H_{11}O_2N$ requires C, 69·8; H, 5·8; N, 7·4%). Deacetylation of this acetate gave *5-hydroxy-2-methylindole* (yield, 80%) which formed colourless, elongated, slender needles, m. p. 133—134°, giving a brownish-purple colouration with alcoholic ferric chloride and a very intense red colouration with Ehrlich's reagent in the cold (Found : C, 73·6; H, 6·0; N, 9·5. C_9H_9ON requires C, 73·5; H, 6·1; N, 9·5%). The *picrate* crystallised from benzene in slender red needles, m. p. 157—158° (decomp.) (Found : N, 14·4. $C_9H_9ON,C_6H_3O_7N_3$ requires N, 14·9%).

5-Nitro-3-hydroxycoumarin (IX).—Interaction of 6-nitrosalicylaldehyde (Ashley, Perkin, and Robinson, *J.*, 1930, 395) (1 g.), aceturic acid (0·8 g.), sodium acetate (0·8 g.), and acetic anhydride (3 ml.) on the steam-bath during 1 hour, with subsequent addition of water, gave a flocculent precipitate of *5-keto-4-(2′-nitro-6′-acetoxybenzylidene)-2-methyl-4 : 5-dihydro-oxazole*, forming yellow needles (1·3 g.), m. p. 180°, from alcohol (Found : C, 54·0; H, 3·5; N, 9·6. $C_{13}H_{10}O_6N_2$ requires C, 53·8; H, 3·5; N, 9·7%).

Hydrolysis of the azlactone (1 g.) with 1·5N-hydrochloric acid (35 ml.) on the steam-bath for 6 hours furnished *5-nitro-3-hydroxycoumarin* which separated from the cooled mixture as a yellow flocculent precipitate and then crystallised from benzene–light petroleum (b. p. 60—80°) in pale yellow needles (0·49 g.), m. p. 206—207° (Found : C, 52·4; H, 2·2; N, 6·4. $C_9H_5O_5N$ requires C, 52·2; H, 2·4; N, 6·8%). This substance gave a 2 : 4-dinitrophenylhydrazone which separated from acetic acid in slender orange needles which melted above 250°.

6-Nitro-2-benzyloxytoluene, prepared by the potassium carbonate method, forming needles, m. p. 62°, from aqueous alcohol (Found : N, 5·7%).

Stability of Hydroxyindoles.—Absolutely pure specimens of the monohydroxyindoles are relatively stable in air, and, with the exception of 4-hydroxyindole, in aqueous solution. A dilute aqueous solution of the latter indole deposits a bluish precipitate in the course of 12 hours. In 1—2% aqueous sodium hydroxide 4- and 5-hydroxyindole give greenish-blue and pale reddish colourations which change respectively to deep blue-green and deep red-purple in the course of 20 minutes. The colourless dilute alkaline solutions of 5-hydroxy-2-methyl- and 6-hydroxy-indole assume faint green and pale bluish tints respectively in the course of 20 minutes. On being kept for longer periods all the alkaline solutions become darker and then finally change to dark brown.

1′ : 2″-*Dinitro-6″-hydroxy-2-phenyl-5 : 6-benz-1 : 3-oxazine* (XIV).—On being kept, a mixture of 2-nitro-6-hydroxybenzaldehyde (1 g.), acetic acid (0·5 ml.), ammonium acetate (0·5 g.), and alcohol (5 ml.) gradually deposited the *oxazine* in long orange needles (0·85 g.) which on recrystallisation from alcohol formed orange yellow cubes, m. p. 204—205°, after sintering at 200° (Found : C, 53·3; H, 2·9; N, 13·1. $C_{14}H_8O_8N_2$ requires C, 53·3; H, 2·9; N, 13·3%). This compound is slightly soluble in cold and readily soluble in warm aqueous sodium hydroxide forming a yellow solution, and with alcoholic ferric chloride gives an orange colouration slowly changing to red. On being refluxed with sodium acetate (2 g.) and acetic anhydride (5 ml.) for 1 hour the metoxazine (1 g.) gave the *acetate* which formed glistening needles (1·1 g.), m. p. 164°, from alcohol (Found : C, 54·0; H, 3·3; N, 11·5. $C_{16}H_{11}O_7N_2$ requires C, 53·8; H, 3·1; N, 11·8%).

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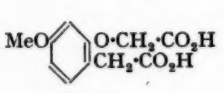
325. *Experiments on the Synthesis of Rotenone and its Derivatives. Part XV.*

By James H. Richards, Alexander Robertson, and Joseph Ward.

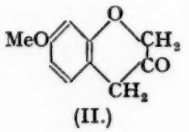
The synthesis of 7- and 8-methoxychroman-3-one by the cyclisation of acids of the type (I) is described.

Condensation of β-chloropropionyl chloride and resorcinol by means of aluminium chloride gave ω-chlororespropiophenone and a compound believed to be ω-hydroxyrespropiophenone, but, when crotonyl chloride was used, 7-hydroxy-2-methylchroman-4-one (VI) was obtained together with a hydroxy-ketone (VII) which on cyclisation gave (VI).

The method developed for the synthesis of chromeno(3′ : 4′ : 2 : 3)chromones of the dehydrorotenone type (Part III, *J.*, 1933, 489), involving the application of the Hoesch reaction in the preparation of intermediate keto-acids of the derrisic acid type, is limited with regard to the phenols which can be employed, *e.g.*, methyl 3-methoxy-2-cyanomethylphenoxyacetate fails to condense with tubanol or dihydrotubanol, whilst with 5 : 7-dihydroxy-2 : 2-dimethylchroman the acyl group enters the 8-position of the chroman residue and not the required 6-position (unpublished results from this laboratory). Accordingly, in the course of a search for suitable alternate routes to the required chromenochromones we prepared examples of the hitherto unknown chroman-3-ones with a view to condensing them with suitably protected ethyl *o*-hydroxybenzoates in order to obtain compounds of the type (III) which would be expected to undergo cyclisation to chromano(3′ : 4′ : 2 : 3)chromones.



(I.)



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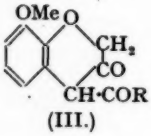

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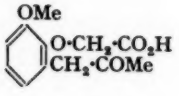
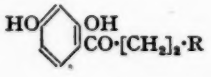


(IV.)

Cyclisation of 5-methoxy-2-carboxymethylphenoxyacetic acid (I) with boiling acetic anhydride containing sodium acetate gave the O-*acetyl* derivative of the enolic form of (II) which on deacetylation furnished the *chroman-3-one* (II). When subjected to similar treatment, 2-methoxy-6-carboxymethylphenoxyacetic acid yielded a mixed product which was resolved into 8-*methoxychroman-3-one* and a C-*acetyl* derivative considered to be (III; R = Me) since it gave a strong ferric reaction and on hydrolysis a *keto-acid* believed to have formula (IV). Attempts to condense 7- or 8-methoxychroman-3-one with ethyl benzoate or ethyl *o*-benzyloxybenzoate under a variety of conditions were unsuccessful; only small amounts of indefinite products were obtained.

In connection with the synthesis of hydroxy-2 : 2-dimethylchroman-4-ones (Parts XII and XIII, *J.*, 1937, 279, 1530) by the Friedel–Crafts route, the condensation of β-chloropropionyl and of crotonyl chloride with resorcinol under the same conditions has been examined. ω-*Chlororespropiophenone* (V; R = Cl) and a substance which appears to be ω-*hydroxyrespropiophenone* (V; R = OH) were isolated from the resinous product formed in the condensation with β-chloropropionyl chloride, but formation of 7-hydroxychromanone or the isomeric coumaranone did not appear to take place.



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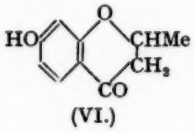

(VI.)

(VII.)

On the other hand crotonyl chloride gave 7-*hydroxy*-2-*methylchroman*-4-*one* (VI) together with a ketone which in all probability has formula (VII) since on cyclisation it is quantitatively converted into the isomeric chroman-4-one. That the latter is the chroman-4-one and not the isomeric 6-hydroxy-2-ethylcoumaranone is proved by the fact that the *methyl ether* readily condenses with *o*-vanillin giving 7′ : 8-dimethoxy-2′-methylchromeno(4′ : 3′ : 2 : 3)benzopyrylium chloride which was characterised by the formation of the *perchlorate* and the *ferrichloride*.

7 : 8-*Dihydroxy*-2 : 2-*dimethylchroman*-4-*one* was prepared by the general method but could not be reduced to the corresponding chroman by Clemmensen's procedure.

Experimental.

7-Methoxychroman-3-one (II).—A mixture of 5-methoxy-2-carboxymethylphenoxyacetic acid (Robertson *et al.*, *J.*, 1936, 422) (5 g.), sodium acetate (7 g.), and acetic anhydride (20 ml.) was gently refluxed for 20 minutes, cooled, and treated with water. 2 Days later the solid (4·3 g.) was collected, washed, dried, and crystallised from light petroleum (b. p. 60—80°), yielding *7-methoxy-3-acetoxy-*Δ^3*-chromen* in colourless rectangular prisms (3 g.), m. p. 97°, soluble in alcohol, benzene, or ethyl acetate, and having a negative ferric reaction (Found : C, 65·7; H, 5·5. $C_{12}H_{12}O_4$ requires C, 65·5; H, 5·5%). 8% Aqueous sodium hydroxide (100 ml.) was added to a solution of this compound (2 g.) in alcohol (150 ml.) kept at 15°, and 10 minutes later the red solution was acidified with hydrochloric acid and extracted with ether (10 × 100 ml.). After having been washed with aqueous sodium hydrogen carbonate (2 × 200 ml.) and water (2 × 200 ml.) and then dried, the combined extracts were evaporated, leaving a red-brown oil which slowly solidified and was triturated with a little ether. Crystallisation of the resulting solid from benzene gave the *chroman-3-one* (II) in diamond-shaped plates (1·1 g.), m. p. 163°, insoluble in cold aqueous sodium hydroxide and having a negative ferric reaction (Found : C, 67·7; H, 5·7. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·6%). The 2 : 4-*dinitrophenylhydrazone* formed orange prisms, m. p. 186°, from alcohol (Found : N, 15·2. $C_{16}H_{14}O_6N_4$ requires N, 15·6%).

8-Methoxychroman-3-one.—Cyclisation of 2-methoxy-6-carboxymethylphenoxyacetic acid (20 g.) by the procedure employed in the case of 5-methoxy-2-carboxymethylphenoxyacetic acid gave a viscous oil (11 g.), b. p. 128°/0·8 mm., which was dissolved in 2·5% methyl-alcoholic sodium hydroxide (80 ml.) kept at 0°. 5 Minutes later the mixture was acidified with 10% acetic acid and extracted with ether, and the dried ethereal extracts evaporated, leaving the product as a pale yellow oil which partly solidified and was repeatedly extracted with light petroleum (b. p. 60—80°). Evaporation of the combined extracts left crystalline material (8·4 g.) with a purple ferric reaction which appeared to be a mixture and was repeatedly triturated with saturated aqueous sodium hydrogen carbonate until a sample did not give a ferric reaction. Crystallised from light petroleum (b. p. 60—80°), the residual solid (5·6 g.) gave the *chroman-3-one* in colourless needles, soluble in alcohol, benzene, or ethyl acetate (Found : C, 67·6; H, 5·6. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·6%). The 2 : 4-*dinitrophenylhydrazone* formed orange prisms, m. p. 192°, from much alcohol (Found : C, 53·5; H, 4·0; N, 15·3. $C_{16}H_{14}O_6N_4$ requires C, 53·6; H, 4·0; N, 15·6%).

Acidification of the combined aqueous sodium hydrogen carbonate washings with 10% acetic acid gave *8-methoxy-4-acetylchroman-3-one* (III; R = Me) (2·7 g.) which separated from light petroleum (b. p. 60—80°) in hexagonal plates, m. p. 108°, exhibiting a purple ferric reaction in alcohol and giving a crystalline precipitate with alcoholic 2 : 4-dinitrophenylhydrazine hydrochloride (Found : C, 65·5; H, 5·5. $C_{12}H_{12}O_4$ requires C, 65·5; H, 5·5%). A solution of this chromanone derivative (1 g.) in 6% aqueous sodium hydroxide (25 ml.) was heated on the steam-bath for 45 minutes, cooled, acidified with hydrochloric acid, and extracted with ether (5 × 120 ml.). Evaporation of the combined, dried extracts left *2-methoxy-6-acetoxylphenoxyacetic acid* (IV) which formed rectangular prisms, m. p. 92°, from light petroleum, having a negative ferric reaction and forming a crystalline precipitate with 2 : 4-dinitrophenylhydrazine solution [Found : C, 60·7; H, 6·0; OMe, 12·8. $C_{11}H_{11}O_4$(OMe) requires C, 60·7; H, 5·9; OMe, 13·0%].

When the thorium salt of 2-methoxyphenoxyacetic acid (from 6 g. of acid) was gradually heated to 350° in a high vacuum a brown oil distilled at 290—320°. Evaporation of a light petroleum extract of this material left a product (0·9 g.) which partly solidified and gave the 2 : 4-dinitrophenylhydrazone (0·8 g.) of 8-methoxychroman-3-one, m. p. 192°, after purification (Found : C, 53·6; H, 4·0; N, 15·7%).

Condensation of β-Chloropropionyl Chloride with Resorcinol.—To a mixture of resorcinol (11 g.), β-chloropropionyl chloride (11·7 g.), and nitrobenzene (100 ml.), kept at room temperature, aluminium chloride (15 g.) was gradually introduced followed 5 days later by ice (100 g.) and concentrated hydrochloric acid (30 c.c.). An ethereal solution of the product and nitrobenzene was washed successively with aqueous sodium hydrogen carbonate, 4% hydrochloric acid, and water. After evaporation of the ether the nitrobenzene was removed with steam and the hot aqueous liquor decanted from viscous resin. On cooling, the liquor deposited *ω-chlororespropiophenone* (V; R = Cl), together with traces of (V; R = OH). The former ketone formed blunt prisms, m. p. 97°, from benzene–light petroleum (b. p. 60—80°), readily soluble in chloroform, alcohol, or benzene, and having an intense purple ferric reaction (Found : C, 53·8; H, 4·9; Cl, 15·1. $C_9H_9O_3Cl$ requires C, 53·8; H, 4·5; Cl, 17·7%). On being heated at 100° this substance decomposed with the evolution of hydrogen chloride.

When the condensation was effected at room temperature for 2 days and then at 130° for 2 hours, the resulting crystallisable material consisted mainly of *ω-hydroxyrespropiophenone* (V; R = OH) which formed long yellow needles, m. p. 91—92°, from dilute alcohol, having an intense purple ferric reaction and exhibiting ketonic properties (Found : C, 59·3; H, 5·5. $C_9H_{10}O_4$ requires C, 59·3; H, 5·5%).

7-Hydroxy-2-methylchroman-4-one (VI).—Interaction of resorcinol (12 g.), crotonyl chloride (10·5 g.), and aluminium chloride (15 g.) in nitrobenzene (80 ml.) at room temperature for 4 days gave on isolation a mixture which was separated into two compounds by means of warm benzene. Crystallisation of the benzene-soluble material from alcohol gave *7-hydroxy-2-methylchroman-4-one* in colourless plates, m. p. 177°, soluble in acetone, alcohol, or chloroform and having a negative ferric reaction (Found : C, 67·3; H, 5·7. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·6%). This substance gave a 2 : 4-*dinitrophenylhydrazone*, forming dark red rhombic prisms, m. p. 259° (decomp.), from ethyl acetate (Found : N, 15·4. $C_{16}H_{14}O_6N_4$ requires N, 15·6%).

The compound insoluble in warm benzene was purified by sublimation in a high vacuum at 85° and obtained in yellow prisms, m. p. 117°, having an intense purple ferric reaction in alcohol and exhibiting ketonic properties (Found : C, 67·3; H, 5·6. $C_{10}H_{10}O_3$ requires C, 67·4; H, 5·6%). On being heated to 200° this *ketone* was converted quantitatively into 7-hydroxy-2-methylchroman-4-one, m. p. 173°, identified by comparison with an authentic specimen. The same conversion was effected when the ketone (0·25 g.) was boiled with 2N-hydrochloric acid (25 ml.) containing alcohol (5 ml.) for 2 hours, and on isolation with ether the product (0·24 g.) had m. p. and mixed m. p. 177° after purification from alcohol.

Methylation of 7-hydroxy-2-methylchroman-4-one by the methyl iodide–potassium carbonate method gave the *ether* which crystallised from dilute alcohol in diamond-shaped plates, m. p. 77°, soluble in the usual organic solvents and having a negative ferric reaction [Found: C, 68·8; H, 6·3; OMe, 15·8. $C_{10}H_9O_2(OMe)$ requires C, 68·7; H, 6·3; OMe, 16·1%]. The 2 : 4-*dinitrophenylhydrazone* separated from ethyl acetate in red needles, m. p. 250° (decomp.) (Found: N, 15·1. $C_{17}H_{16}O_6N_4$ requires N, 15·2%). Crystallised from alcohol or light petroleum–dioxan, the *semicarbazone* formed rhombic prisms, m. p. 218—219° (Found: C, 57·9; H, 6·0; N, 17·0. $C_{12}H_{15}O_2N_3$ requires C, 57·8; H, 6·0; N, 16·9%). The *thiosemicarbazone* was obtained in needles, m. p. 239° (decomp.), from alcohol (Found: C, 54·4; H, 5·8; N, 15·8. $C_{12}H_{15}O_2N_3S$ requires C, 54·3; H, 5·7; N, 15·9%), and the *oxime* in needles, m. p. 161°, from the same solvent (Found: N, 6·8. $C_{11}H_{13}O_3N$ requires N, 6·8%). Carefully purified specimens of the oxime, semicarbazone, and thiosemicarbazone gave a ferric reaction in alcohol.

A solution of 7-methoxy-2-methylchroman-4-one (0·1 g.) and *o*-vanillin (0·1 g.) in ethyl acetate (5 ml.) saturated with hydrogen chloride was kept for 3 days and the resulting chloride collected, washed with ether, and crystallised from 8% hydrochloric acid, forming red needles. The *perchlorate* separated from acetic acid in tiny orange needles, decomposing at above 280° (Found: C, 55·6; H, 4·4; Cl, 8·3. $C_{19}H_{17}O_8Cl$ requires C, 55·8; H, 4·2; Cl, 8·7%), and the *ferrichloride* formed small red-brown prisms, m. p. 199°, (decomp.), from acetic acid (Found: C, 45·5; H, 3·3. $C_{19}H_{17}O_4Cl_4Fe$ requires C, 45·0; H, 3·4%).

7 : 8-*Dihydroxy*-2 : 2-*dimethylchroman*-4-*one*.—After gradual addition of powdered aluminium chloride (17 g.) to a solution of $\beta\beta$-dimethylacrylyl chloride (11·7 g.) and pyrogallol (15 g.) in nitrobenzene (100 ml.), the mixture was kept at room temperature for 5 days, then treated with ice (150 g.) and dilute hydrochloric acid (100 c.c.), and extracted several times with ether. The combined ethereal extracts were washed with water and evaporated, and, after the removal of the nitrobenzene by a current of steam, the hot liquor was decanted from a brown viscous resin. On cooling, the *chromanone* separated in rhombic prisms; a further quantity of product was obtained by boiling the resin with the residual liquor from the crystalline material (yield, 5 g.). Recrystallised from dilute alcohol or benzene–light petroleum (b. p. 60—80°), the compound formed yellow rhombic prisms, m. p. 142—143°, having a brownish-purple ferric reaction in alcohol (Found: C, 63·5; H, 5·8. $C_{11}H_{12}O_4$ requires C, 63·5; H, 5·8%). The 2 : 4-dinitrophenylhydrazone formed orange-red needles, m. p. 259° (decomp.), from alcohol. Methylation of the foregoing chromanone (0·5 g.) with methyl iodide (1 ml.) and potassium carbonate (1·5 g.) in boiling acetone for 6 hours with the addition of more iodide (1 ml.) and carbonate (1 g.) after 2 hours gave 7 : 8-*dimethoxy*-2 : 2-*dimethylchroman*-4-*one* which separated from dilute alcohol in colourless blunt prisms, m. p. 76° [Found: OMe, 26·2. $C_{11}H_{10}O_2(OMe)_2$ requires OMe, 26·3%].

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326. *Derivatives of* 2 : 4 : 6-*Trinitrotoluene:* (a) *the Nitration of Polynitrostilbenes,* (b) 2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl Alkyl* (*Aryl*) *Sulphides.*

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The nitration of 2 : 4 : 6-tri- and 2 : 4 : 6 : 2′-tetranitrostilbene gives 2 : 4 : 6 : 2′ : 4′-pentanitrobenzil. In the case of the 2 : 4 : 6-derivative, 2 : 4-dinitro- and 2 : 4 : 6-trinitro-benzoic acid are also formed. The pentanitrobenzil is also obtained from 2 : 4 : 6 : 2′ : 4′-pentanitrostilbene under similar conditions. 2-(2 : 4 : 6-Trinitrophenyl)ethanol yields the chloride, and this with the sodium derivative of the appropriate thiol gives methyl and *p*-tolyl 2-(2 : 4 : 6-trinitrophenyl)ethyl sulphide and thence the sulphones. The chloride forms quaternary salts with pyridine and quinoline.

WHEN 2 : 4 : 6-trinitrostilbene (Bishop and Brady, *J.*, 1922, **121**, 2367) is heated with sulphuric acid and nitric acid (*d* 1·41), 2 : 4 : 6 : 2′ : 4′-*pentanitrostilbene* is formed. An attempt to synthesise this compound from 2 : 4 : 6-trinitrotoluene and 2 : 4-dinitrobenzaldehyde failed. When this or the 2 : 4 : 6-trinitro- or the 2 : 4 : 6 : 2′-tetranitro-derivative (Bishop and Brady, *loc. cit.*) is heated with sulphuric acid and nitric acid (*d* 1·5) at 100°, oxidation occurs and a compound (*D*, see p. 1613) is obtained which appears to be 2 : 4 : 6 : 2′ : 4′-*pentanitrobenzil* because oxidation with hydrogen peroxide in glacial acetic acid gives 2 : 4-dinitrobenzoic acid and 1 : 3 : 5-trinitrobenzene. The last-named compound arises by decarboxylation of 2 : 4 : 6-trinitrobenzoic acid. Pentanitrobenzil also gives 2 : 4-dinitrobenzoic acid on oxidation with alkaline potassium permanganate. After the formation of pentanitrobenzil from 2 : 4 : 6-trinitrostilbene, 2 : 4-dinitro- and 2 : 4 : 6-trinitro-benzoic acid can be isolated from the spent acids. These probably arise from oxidation at an intermediate stage in the formation of *D*, as this can be recovered quantitatively from a hot mixture of sulphuric and fuming nitric acid.

2 : 4 : 6 : 2′ : 4′-Pentanitrobenzil (*D*) is colourless, whereas mono- and di-nitrobenzil are yellow. It turns orange-brown in light. Some dinitrobenzils change from pale yellow to green under similar conditions (see p. 1614).

Attempts to prepare pentanitrobenzil by the nitration of 2 : 2′-dinitrobenzil (Kliegl and Haas, *Ber.*, 1911, **44**, 1213; Chattaway and Coulson, *J.*, 1928, 1363) or 4 : 4′-dinitrobenzil (Chattaway and Coulson, *loc. cit.*) failed. When the 4 : 4′-derivative was heated with sulphuric and fuming nitric acid under conditions which converted trinitrostilbene to pentanitrobenzil, only 2 : 4-dinitrobenzoic acid was obtained. With sulphuric and nitric acid (*d* 1·41) the dinitrobenzil was unchanged. The 2 : 2′-dinitro-derivative with sulphuric and fuming nitric acid under similar conditions was mostly unaltered.

The pentanitro-derivatives of diphenylacetylene, deoxybenzoin, hydrobenzoin, and benzoin possess the same carbon skeleton as pentanitrobenzil, and could be oxidised to the corresponding di- and tri-nitrobenzoic acids. It would appear improbable, however, that *D* can be identical with one of these compounds, as they would all probably be oxidised to the benzil derivative with hot nitrating mixture.

During the purification of the pentanitrobenzil a nitro-compound, m. p. 260°, which gives a deep purple colouration with acetone and sodium hydroxide, was isolated. This has been detected by other workers during the nitration of various aromatic hydrocarbons and will be described later.

2-(2 : 4 : 6-Trinitrophenyl)ethanol yields the *chloride* with phosphorus pentachloride. This with methylthiol and *p*-tolylthiol in presence of sodium ethoxide gives *methyl* 2-(2 : 4 : 6-*trinitrophenyl*)*ethyl sulphide* (*A*) and p-*tolyl* 2-(2 : 4 : 6-*trinitrophenyl*)*ethyl sulphide* (*B*). From *A* the corresponding *sulphone* and from *B* the *sulphoxide* and *sulphone* were prepared by oxidation with hydrogen peroxide in glacial acetic acid. *p*-Tolyl 2-(2 : 4 : 6-trinitrophenyl)ethyl sulphone was also obtained from the nitrated chloride and sodium toluene-*p*-sulphinate.

The chloride forms quaternary salts with pyridine and quinoline; 2-(2 : 4 : 6-*trinitrophenyl*) *ethylpyridinium chloride*, *picrate*, and *benzenesulphonate* were prepared and also the *quinolinium chloride*.

Experimental.

Nitration of 2 : 4 : 6-*Trinitrostilbene.*—The nitro-compound (7·5 g.) was gradually added to a mixture of sulphuric acid (50 c.c.) and nitric acid (*d* 1·5; 50 c.c.) and heated for 8 hours at 100°. The experiment was repeated at 120—130° for 3 hours. In each case effervescence occurred and a crystalline solid (*A*) separated after 10 minutes but was not removed. The nitration mixtures were poured on ice giving identical buff-coloured solids (*B*) of m. p. 240—250° (decomp.). Other nitrations at 100°, during 4 and 8 hours respectively, gave similar results. The spent acids (*S*) were retained.

Products *B* were united and washed with aqueous sodium carbonate which became dark red. The insoluble matter (*C*, 4·2 g.) was separated; m. p. 220—240° (decomp.). Extraction with acetone left a sparingly soluble product (*D*), m. p. 260° (decomp.). The acetone extract (*E*) was reserved. Substance *D* (2 : 4 : 6 : 2′ : 4′-*pentanitrobenzil*) was crystallised from acetone (m. p. 261°), and then successively from glacial acetic acid, acetone–light petroleum, and benzene–light petroleum (m. p. 260° each time). Another specimen was crystallised 7 times from various similar mixtures of solvents, and the m. p. 260° (decomp.) confirmed. Further nitration under the same conditions as before did not alter the m. p. (Found: C, 39·2, 39·4; H, 1·5, 1·4; N, 15·9, 16·1; *M*, 453. $C_{14}H_6O_{12}N_6$ requires C, 38·6; H, 1·2; N, 16·1%; *M*, 435).

The solid *A* when removed after 15 minutes melted at 260° (decomp.) on recrystallisation and was identical with *D*. Long heating is, therefore, probably unnecessary.

The spent acids *S* gave a deep red colour with sodium hydroxide. On concentration a solid was deposited. This was washed with nitric acid and dried; m. p. 170—220°. On recrystallisation from benzene it was identified as 2 : 4 : 6-trinitrobenzoic acid by the blood-red colouration with sodium carbonate and by m. p. (*a*) 210°, (*b*) 212°, and mixed m. p. (*a*) 213° and (*b*) 212° with an authentic specimen of m. p. 210°. The m. p. depends on the rate of heating. The benzene mother liquor with light petroleum yielded a solid which after three crystallisations had m. p. 162—166°. Trinitrobenzoic acid was removed from this as the insoluble barium salt. The soluble portion, on acidification, gave an acid, m. p. 166°, which on crystallisation had m. p. and mixed m. p. 179—180° with authentic 2 : 4-dinitrobenzoic acid. The *p*-nitrobenzyl ester had m. p. and mixed m. p. 137° with an authentic specimen m. p. 139°.

In order to check the purity of the pentanitrobenzil, attempts were made to prepare addition products with naphthalene and anthracene. Yellow and red solutions in acetone were obtained respectively, but only pentanitrobenzil separated on concentration.

More definite evidence of compound formation was obtained with solid thiophthen [thiopheno (2′ : 3′ : 3 : 2)thiophen] (Challenger and Harrison, *J. Inst. Pet. Tech.*, 1935, **21**, 135; Challenger and Gibson, *J.*, 1940, 305). When pentanitrobenzil (1 mol.) and thiophthen, m. p. 54° (4—5 mols.), were dissolved in acetone and the solution completely evaporated, an impure yellow solid separated which, after being washed with a little acetone, could be recrystallised with only partial decomposition from acetone containing thiophthen. This operation was repeated thrice, the m. p. of the impure addition product being 200—220°, 220—230°, and 220—224°. Treatment in acetone solution with ligroin regenerated pentanitrobenzil, the m. p. of which could not be raised above 259° (Found: C, 39·1, 39·1; H, 1·2, 1·2; N, 15·8, 15·9%). By heating the yellow addition product for 10 minutes at 110° the thiophthen volatilised and the residue melted at 258—259°.

Pentanitrobenzil was recovered largely unchanged after being boiled with hydroxylamine hydrochloride in glacial acetic acid or added to *o*-phenylenediamine in hot glacial acetic acid.

Nitration of 2 : 4 : 6 : 2′-*Tetranitrostilbene.*—The tetranitro-compound was prepared by Bishop and Brady's method (*J.*, 1922, **121**, 2367); m. p. 181°. 0·5 G. was nitrated under the conditions employed for the trinitro-derivative. A crystalline deposit separated after 10 minutes but was not removed. After 3 hours the mixture was filtered through glass wool and the solid washed with nitric acid, water, sodium carbonate, and water, and dried; m. p. 256° (decomp.). On recrystallisation from acetone–light petroleum, the m. p. and mixed m. p. was 258° with pentanitrobenzil of m. p. 259°. The spent acids contained some trinitrobenzoic acid and yielded a small amount of a compound, m. p. 260°, which gave a purple colour with acetone and sodium hydroxide. This was also obtained on acidification of the sodium carbonate washings of *B*—see the nitration of trinitrostilbene, p. 1613.

Nitration of 2 : 4 : 6-*Trinitrostilbene under Milder Conditions.*—The nitro-compound (5 g.) was gradually added to a mixture of sulphuric acid (10 c.c.) and nitric acid (*d* 1·41; 10 c.c.). An orange solid separated. The mixture was heated for 2 hours at 100° and poured on ice. The crude solid (4·5 g.) on recrystallisation from benzene or from acetone–light petroleum and finally from benzene–acetone, formed greenish-yellow needles of 2 : 4 : 6 : 2′ : 4′-*pentanitrostilbene*, m. p. 198—199° (Found: C, 41·6, 41·6; H, 1·8, 1·85; N, 16·8, 17·0. $C_{14}H_7O_{10}N_5$ requires C, 41·5; H, 1·74; N, 17·3%). A later preparation gave 21 g. of crude product from 18 g. of trinitrostilbene.

Oxidation of 2 : 4 : 6 : 2′ : 4′-*pentanitrostilbene to* 2 : 4 : 6 : 2′ : 4′-*pentanitrobenzil.*—The pentanitrostilbene (2 g.) was heated with the nitration mixture as in the case of the tetranitro-compound. A solid separated after 10 minutes. Pentanitrobenzil (1 g.), m. p. and mixed m. p. 259°, was isolated as before. The spent acids in this and the two preceding nitrations gave a deep red colour with sodium hydroxide.

Orientation of the Nitro-groups in Pentanitrobenzil.—(*a*) The nitro-compound (2 g.) was dissolved in boiling glacial acetic acid, and hydrogen peroxide (20 vols.) added at intervals until a test-portion, on dilution with water, showed only a faint turbidity. The solution was then diluted with water, filtered, and evaporated to dryness. The yellow residue was extracted with hot benzene, which deposited a solid. This, on recrystallisation from benzene, had m. p. and mixed m. p. 180° with authentic 2 : 4-dinitrobenzoic acid. Yield, 0·66 g.

The mother liquors yielded 1 : 3 : 5-trinitrobenzene which, after being washed with sodium hydrogen carbonate and recrystallised from alcohol, had m. p. 119° and 120° in admixture with an authentic specimen, m. p. 122°. The naphthalene and anthracene compounds melted at 153—154° and 162—163° respectively (lit. gives 152·5° and 164°). Trinitrobenzoic acid was not isolated, although the crude oxidation product gave a red colour with sodium hydroxide. Oxidation of 2 : 4 : 6-trinitrobenzoic acid under these conditions gave trinitrobenzene.

(*b*) The pentanitrobenzil (0·5 g.) was boiled with excess of alkaline 3% aqueous potassium permanganate till oxidation ceased. Addition of a few drops of alcohol and filtration gave a pale yellow solution (absence of trinitrobenzoic acid) which, on acidification and extraction with ether, yielded 2 : 4-dinitrobenzoic acid, m. p. 177—178°, mixed m. p. 179° with a specimen of m. p. 180°. The *p*-nitrobenzyl ester had m. p. and mixed m. p. 140°.

Properties of Pentanitrobenzil.—The colourless compound gives no colour with aqueous sodium carbonate, in which it is insoluble. With aqueous sodium hydroxide it gives slowly a faint orange colour which becomes brown on standing or on boiling, and the solution then contains nitrite or nitrate. Ammonia is evolved on boiling. On exposure to light the colourless compound becomes orange red, and the m. p. falls slightly (to 257—258°). 2 : 2′- and 2 : 3′-Dinitrobenzil are phototropic (Chattaway and Coulson, *J.*, 1926, 1073).

The compound was recovered unchanged after treatment with a chloroform solution of bromine at the b. p. and evaporation of the solvent. With aqueous sodium dichromate and sulphuric acid at 150° some oxidation occurred, but even after several hours the reaction was incomplete, and unchanged 2 : 4 : 6 : 2′ : 4′-pentanitrobenzil was recovered. Chattaway and Coulson found that 2 : 2′-dinitrobenzil does not form a quinoxaline, and is the only one of the mono- and di-nitrobenzils which is not readily oxidised by chromic acid (*J.*, 1928, 1081). On being heated with nitric acid (*d* 1·5) at 100° or with this acid and sulphuric acid at 130—135°, pentanitrobenzil was recovered almost quantitatively. The evaporated nitric acid contained no trinitrobenzoic acid.

2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl chloride.*—2-(2 : 4 : 6-Trinitrophenyl)ethanol (Vender, *Gazzetta*, 1915, **45**, II, 97; Bruson and Butler, *J. Amer. Chem. Soc.*, 1946, **68**, 2348) was largely recovered unchanged after (*a*) treatment with excess of thionyl chloride at room temperature for 10 days, (*b*) being boiled with excess of thionyl chloride under reflux for several hours, or (*c*) treatment with anhydrous hydrogen chloride in glacial acetic acid. In each case the mixture was poured into water or on ice and the product crystallised from benzene or aqueous alcohol.

The finely ground trinitrophenylethanol (5 g.) was covered with light petroleum (b. p. 40—60°) and treated with finely ground phosphorus pentachloride (7 g.) under a reflux condenser and calcium chloride tube. Reaction began on slightly warming; when it slackened a further 0·5 g. of the pentachloride was added. The mixture was finally poured on ice, the solvent volatilised, and the residue crystallised 3 or 4 times from benzene–light petroleum or carbon tetrachloride, giving colourless needles, m. p. 76°, which became opaque when dry. Yield 2·4 g. of m. p. 72°. Later experiments gave 8 g. from 10 g. of the ethanol. The optimum quantity of ethanol appears to be 10—20 g. With phosphorus oxychloride as solvent instead of light petroleum the yield was 1·7 g. of *chloride*, m. p. 67°, from 5 g. of ethanol (Found: C, 35·2; H, 2·2; Cl, 13·4. $C_8H_6O_6N_3Cl$ requires C, 34·8; H, 2·2; Cl, 12·9%).

Attempts to convert the chloride into the thiocyanate or selenocyanate by reaction with the corresponding sodium or potassium salts in acetone were unsuccessful. No reaction occurred with silver cyanide or thiocyanate in cold or boiling benzene. 2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl pyridinium chloride* was prepared from the chloride in pyridine. A solid separated after a few hours. After being washed with acetone and crystallised from aqueous acetone or alcohol–ether this melted at 132—134° (Found: Cl, 9·8, 10·0. $C_{13}H_{11}O_6N_4Cl$ requires Cl, 10·0%). The corresponding *picrate* on crystallisation from aqueous acetone had m. p. 115—116° depending slightly on the rate of heating (Found, by Nitron method: $-O\cdot C_6H_2(NO_2)_3$, 39·1, 39·7. $C_{19}H_{13}O_{13}N_7$ requires 41·6%). A slight trace of some halogen compound could not be removed on crystallisation. The *benzenesulphonate* formed a white precipitate,

m. p. 181—182° after crystallisation from alcohol–ether, 183° after crystallisation from water (Found: C, 48·2; H, 3·5; N, 11·7. $C_{19}H_{16}O_9N_4S$ requires C, 47·8; H, 3·4; N, 11·8%).

2-(2 : 4 : 6-*Trinitrophenyl*)*ethylquinolinium chloride* was prepared by using the minimum quantity of quinoline; with more than this the quaternary chloride did not separate. 1 G. of the chloride gave 0·4 g. of the quinolinium compound which melted at 159—160° (decomp.) after recrystallisation from alcohol–ether or aqueous acetone (Found: Cl, 8·9, 8·9. $C_{17}H_{13}O_6N_4Cl$ requires Cl, 8·75%). Addition of sodium picrate to an aqueous solution gave a precipitate, m. p. 160—180°, m. p. 200° after recrystallisation from alcohol. The mixed m. p. with quinoline picrate was 201°, the arylquinolinium complex having apparently decomposed.

p-*Tolyl* 2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl Sulphide.*—The chloride (1 g.) and *p*-tolylthiol (0·5 g.; slight excess) were dissolved in alcohol, and a solution of sodium (0·07 g., slightly less than 1 atom) in alcohol slowly added. The solution was only faintly red at the conclusion of the addition, and was gently boiled (1 hour) till no more sodium chloride separated. Excess of sodium ethoxide gives rise to red tar. Filtration and concentration gave an oil which solidified and was recrystallised twice from alcohol. The *sulphide* formed orange-yellow needles, m. p. 91° (Found: C, 49·6; H, 3·65; N, 11·3; S, 8·7. $C_{15}H_{13}O_6N_3S$ requires C, 49·6; H, 3·6; N, 11·6; S, 8·8%).

With methyl iodide at room temperature, or on boiling the mixture with nitromethane, no methiodide was formed.

p-*Tolyl* 2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl Sulphoxide.*—The sulphide in glacial acetic acid at 100° was treated with excess of hydrogen peroxide. After 15 minutes, dilution with water gave pale yellow needles of the *sulphoxide*, m. p. 132° raised to 137—138° on recrystallisation from alcohol (Found: C, 47·8; H, 3·25; N, 10·6; S, 8·25. $C_{15}H_{13}O_7N_3S$ requires C, 47·5; H, 3·4; N, 11·1; S, 8·4%).

p-*Tolyl* 2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl Sulphone.*—The sulphide (0·8 g.) was heated at 100° with excess of hydrogen peroxide in glacial acetic acid for 1 hour; a white solid separated (0·7 g.), m. p. 190°. The m. p. rose to 197—198° after 2 crystallisations from acetone and 1 from acetone-light petroleum (Found: C, 45·8; H, 3·6; N, 10·5; S, 8·0. $C_{15}H_{13}O_8N_3S$ requires C, 45·6; H, 3·3; N, 10·6; S, 8·1%). The same *sulphone* was obtained when the chloride (2 g.) and toluene-*p*-sulphinic acid (1 g.) were boiled with alcohol (100 c.c.) and sodium (0·1 g.) in alcohol, slowly added. After 1 hour, sodium chloride and brown crystals separated. These were treated with water, and the insoluble matter (1·1 g.) recrystallised from acetone–alcohol; m. p. and mixed m. p. with the sulphone, 197—198°. A further quantity (0·5 g.) was obtained from the filtered reaction mixture.

Methyl 2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl Sulphide.*—The nitrated chloride (2 g.) in hot alcohol was gradually treated with a solution of methylthiol (0·5 c.c.) and sodium (0·14 g.) in alcohol. After 1 hour's boiling, the filtered solution was concentrated, depositing a brownish-yellow solid, m. p. 85—86° after recrystallisation from alcohol (Found: C, 37·9; H, 3·1; N, 14·6; S, 11·2. $C_9H_9O_6N_3S$ requires C, 37·6; H, 3·1; N, 15·4; S, 10·6%).

Dimethyl-2-(2 : 4 : 6-*trinitrophenyl*)*ethylsulphonium Iodide.*—When the *sulphide* was left for several days with methyl iodide and nitromethane, or when the mixture was boiled for 30 minutes, red needles separated on cooling, m. p. 148° (decomp.). These contained ionic iodine and were recrystallised from warm water; the *iodide* then had m. p. 150° unchanged on further crystallisation, although a slight odour of dimethyl sulphide was apparent. This was much increased by the action of cold, and still more by that of warm, sodium hydroxide (cf. Crane and Rydon, *J.*, 1947, 766) (Found: I, 29·2. $C_{10}H_{12}O_6N_3IS$ requires I, 29·6%).

Methyl 2-(2 : 4 : 6-*Trinitrophenyl*)*ethyl Sulphone.*—The sulphide was heated at 100° in glacial acetic acid with excess of hydrogen peroxide. The white solid *sulphone* which separated was recrystallised from acetone–light petroleum; m. p. 187° constant (Found: C, 33·6; H, 3·0; N, 13·2; S, 10·0; $C_9H_9O_8N_3S$ requires C, 33·9; H, 2·8; N, 13·2; S, 10·0%).

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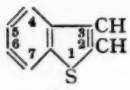
327. *Some Derivatives of Thionaphthen.*

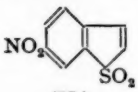
By FREDERICK CHALLENGER and PETER H. CLAPHAM.

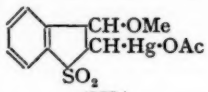
Nitrothionaphthen sulphone is oxidised to 4-nitro-2-sulphobenzoic acid. The nitro-group is therefore in position (6). Mercuration of thionaphthen sulphone in methyl alcohol gives 2-acetoxymercuri-3-methoxy-2 : 3-dihydrothionaphthen sulphone. Methyl and ethyl alcohols in presence of the corresponding sodium alkoxides yield 3-alkoxy-2 : 3-dihydrothionaphthen sulphone, and hydriodic acid gives the corresponding iodo-compound. Dihydrothionaphthen sulphone is obtained by reduction of thionaphthen sulphone with zinc dust and sodium hydroxide.

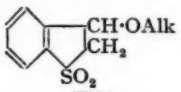
THE destruction of the aromatic system present in the thiophen ring of thionaphthen (I) would be expected to alter the position of substitution. Thionaphthen is nitrated in position 3, but we find that the nitrothionaphthen sulphone prepared by Lanfry (*Compt. rend.*, 1912, **154**, 519) is the 6-*nitro*-derivative (II), oxidation with potassium permanganate giving 4-nitro-2-sulphobenzoic acid which we find is also produced in small amount during the nitration. The acid, its *dimethyl* ester, and its cinchonine salt have been compared with authentic specimens

obtained by oxidation of 4-nitrotoluene-2-sulphonic acid (Kastle, *Amer. Chem. J.*, 1889, **11**, 179; 1910, **44**, 485) during which process *p*-nitrobenzoic acid is also formed.


(I).


(II.)


(III.)


(IV.)

Thionaphthen is mercurated in the 3-position by mercuric acetate and methyl alcohol (Challenger and Miller, *J.*, 1939, 1005) but we find that under comparable conditions the sulphone gives an addition product which is *2-acetoxymercuri-3-methoxy-2 : 3-dihydrothionaphthen sulphone* (III), the $-CH(Hg\cdot OAc)\cdot CH(OMe)-SO_2-$ group exhibiting purely unsaturated properties. The position of the adducts is inferred from the established course of addition to unsaturated sulphones. The reaction is analogous to the well-known addition of mercury salts to ethylene and similar compounds. Where, as in 1 : 2-dihydronaphthalene and anethole the double bond is in the 1 : 2-position to the benzene nucleus, addition of mercuric acetate does not occur, but the compound is oxidised to a glycol (Straus and Lemmel, *Ber.*, 1921, **54**, 36). In the case of thionaphthen sulphone the 2 : 3-double bond is modified by the electrophilic properties of the $-SO_2-$ group. Like divinyl sulphone (Alexander and McCombie, *J.*, 1931, 1913) and benzyl propenyl sulphone (Rothstein, *J.*, 1934, 684), thionaphthen sulphone forms addition *products* (IV) with alcohols in presence of sodium alkoxide and also with hydriodic acid. This was first observed in an attempt to reduce the sulphone with sodium and ethyl alcohol. Dihydrothionaphthen sulphone was, however, obtained by reduction with zinc dust and sodium hydroxide. Direct comparison with a specimen prepared from carefully purified dihydrothionaphthen (regenerated from its mercurichloride) by oxidation with potassium permanganate confirmed the m. p. 92° given by Fricke and Spilker (*Ber.*, 1925, **58**, 1594). It is difficult to explain the figure 98° given by von Braun, Bayer, and Kayser (*ibid.*, p. 2167). In the preparation of dihydrothionaphthen from nitrated phenylethyl chloride (see p. 1617) it is unnecessary to remove the whole of the *p*-compound before reduction and formation of the diazonium xanthate. This greatly simplifies the procedure.

Dihydrothionaphthen and its sulphone appear to give the same mononitro-derivative with nitric acid (*d* 1·5).

Experimental.

Nitration of Thionaphthen Sulphone.—The sulphone was prepared by Lanfry's method (*loc. cit.*; see also Weissgerber and Krüber, *Ber.*, 1920, **53**, 1554). The yield was 12 g. from 10 g. of thionaphthen and the diluted glacial acetic acid–hydrogen peroxide gave only a slight turbidity with barium chloride. The sulphone (10 g.) was gradually added to nitric acid (*d* 1·5; 30 c.c.) cooled by water, and left at room temperature for 3 hours. Pouring into water gave a pinkish-yellow solid (12·5 g., m. p. 184°). After three crystallisations from benzene the m. p. was 188°, unchanged on crystallisation from glacial acetic acid. If the temperature during nitration rose above about 15° only a red tar was obtained. The benzene mother-liquors yielded only the nitrosulphone and a small amount of brown resin (Found : C, 45·9; H, 2·6; N, 6·0. Calc. for $C_8H_5O_4NS$: C, 45·5; H, 2·4; N, 6·6%).

Nitrothionaphthen sulphone dibromide was prepared from its constituents in glacial acetic acid. After 18 hours, dilution with water gave a yellow solid, m. p. 145°, which was recrystallised (charcoal) from aqueous alcohol and from benzene–ligroin; m. p. 149—150° (Found : C, 26·1; H, 1·4; Br, 42·9, $C_8H_5O_4NBr_2S$ requires C, 25·9; H, 1·35; Br, 43·1%).

Oxidation of Nitrothionaphthen Sulphone. Orientation of the Nitro-group.—The nitro-sulphone (5 g.) was suspended in a solution of potassium hydroxide (4 g.) in water (500 c.c.), and solid potassium permanganate (12 g.) gradually added until slight excess was present. This was removed by hydrogen peroxide, and the filtered solution neutralised with dilute hydrochloric acid and concentrated till a neutral potassium salt separated on cooling. The solution was then strongly acidified, whereupon the acid potassium salt of 4-nitro-2-sulphobenzoic acid separated (5·5 g., 76%). This was recrystallised from water, air-dried, and titrated with standard alkali (phenolphthalein) (Found : *M*, 307, 308; loss at 150°, 6·1. Calc. for $C_7H_4O_7NSK,H_2O$: *M*, 303; H_2O, 5·95%).

The neutral potassium salt gave a cinchonine salt (not analysed) which on crystallisation from water had the unsharp but constant m. p. of 165—170° alone and in admixture with a sample of identical m. p. prepared from authentic dipotassium 4-nitro-2-sulphobenzoate.

The acid potassium salt in hot water was treated with barium chloride solution, and neutralised by barium carbonate paste. The salt which separated was suspended in hot water, dissolved by addition of dilute hydrochloric acid, barium removed by sulphuric acid, and the filtrate concentrated till the acid crystallised on cooling. Dried on tile, this had m. p. 70°, and after heating at 80° for several hours, 144°. A few minutes' exposure to air lowered the m. p. by several degrees. A sample of m. p. 142° had mixed m. p. 144° with authentic 4-nitro-2-sulphobenzoic acid, m. p. 145°. A second oxidation of the nitrated sulphone gave a specimen of this acid which had m. p. and mixed m. p. 140—142°, with a known sample of the same m. p. The *dimethyl* ester was prepared by means of diazomethane, and, crystallised from benzene–light petroleum, had m. p. 114—115° alone and when mixed with an authentic sample of the same m. p. (Found : C, 39·4; H, 3·3; S, 11·8. $C_9H_9O_7NS$ requires C, 39·3; H, 3·3; S, 11·6%).

Oxidation during Nitration of Thionaphthen Sulphone.—The spent acid was concentrated, and traces of white solid removed, m. p. 206°. This was insufficient for crystallisation but probably consisted of impure *p*-nitrobenzoic acid resulting from elimination of the sulpho-group from 4-nitro-2-sulphobenzoic acid. On evaporation to a syrup, this sulphonic acid crystallised on cooling. When dried as before it melted at 140° and gave a dimethyl ester, m. p. 114—115°.

Preparation of Authentic 4-Nitro-2-sulphobenzoic Acid and its Dimethyl Ester. Hydrolysis of the Sulpho-group.—The potassium salt of 4-nitrotoluene-2-sulphonic acid was prepared by Kastle's method (*loc. cit.*, 1910) from *p*-nitrotoluene, and this (10 g.) was oxidised by warming with alkaline permanganate. The 4-nitro-2-sulphobenzoic acid was isolated as the acid potassium salt (9·5 g.), which was converted into the free acid by the method already described. This had m. p. about 70° when dried on tile (71·2°; Hausser, *Bull. Soc. chim.*, 1891, **6**, 391), and after several hours' heating at 80° the m. p. was 144° (146°; Hausser, *loc. cit.*). The dimethyl ester, prepared by the action of ethereal diazomethane on the anhydrous acid, had m. p. 114—115° (Found: C, 39·8; H, 3·3; S, 11·8%).

During the concentration of the aqueous solution of the sulphonic acid a small amount of white solid was deposited, m. p. 238°. This was obtained in larger quantity (0·25 g.) when the *alkaline* solution of the acid was concentrated before the isolation of the acid potassium salt. Recrystallised from benzene, it had m. p. 238—239° and mixed m. p. 238—239° with a known specimen of *p*-nitrobenzoic acid of the same m. p. The *p*-nitrobenzyl ester had m. p. 168° and mixed m. p. 168° with an authentic specimen of the same m. p.

Reduction of Thionaphthen Sulphone.—After treatment of the sulphone with zinc dust and acetic acid and with sodium amalgam and water it was recovered unchanged. When finely ground and heated at 100° with excess of zinc dust and aqueous sodium hydroxide, the solid gradually melted to a colourless oil. After 2 hours, water was added and the mixture boiled till the oil dissolved. On filtration and concentration, crystals separated, m. p. 90—91° and 92° on crystallisation from water, unchanged on further crystallisation from water or ligroin (A). The mixed m. p. with authentic dihydrothionaphthen sulphone (B) was 92° (see below) (Found: C, 57·0; H, 4·9; S, 19·0. Calc. for $C_8H_8O_2S$: C, 57·1; H, 4·8; S, 19·0%).

Reactivity of the Double Bond in Thionaphthen Sulphone. Ethoxydihydrothionaphthen Sulphone.—In an earlier attempt to reduce the sulphone, 2·5 g. in ethyl alcohol (50 c.c.) were gradually treated with sodium (1 g.) in thin slices. The orange solution on dilution with water gave 1·8 g. of a white solid, m. p. 106° and 107—108° after crystallisation from water, alcohol, or benzene–petrol. Repeated operations failed to alter the m. p. of the *product* (Found: C, 56·7; H, 5·8; S, 15·5; OEt, by Zeisel, 21·9. $C_{10}H_{12}O_3S$ requires C, 56·6; H, 5·7; S, 15·1; OEt, 21·2%). The slightly high value for ethoxyl is due to the formation of traces of silver sulphide.

Methoxydihydrothionaphthen Sulphone.—When the experiment was repeated but with methyl alcohol as solvent, the *product* melted at 77—78° after crystallisation from water (Found: OMe, 16·4. $C_9H_{10}O_3S$ requires OMe, 15·7%).

Addition of Hydrogen Iodide at the Double Linkage. Iododihydrothionaphthen Sulphone.—The sulphone was warmed with excess of hydriodic acid (*d* 1·9) for 3 hours. Dilution with water gave a dark oil which was well washed with water and solidified on rubbing with alcohol. The *iodo*-compound crystallised from alcohol in colourless needles, m. p. 104—105° (Found: I, 43·3. $C_8H_7O_2IS$ requires I, 43·5%).

Reduction with sodium amalgam and water in the cold gave thionaphthen sulphone, m. p. 142°, and not its dihydro-derivative.

Mercuration of Thionaphthen Sulphone.—The sulphone (2 g.) in methyl alcohol (80 c.c.) was heated for 5 days with a solution of yellow mercuric oxide (8 g.) in glacial acetic acid (10 c.c.) and water (60 c.c.). Some mercurous acetate separated. On filtration, removal of methyl alcohol, and dilution with water, a solid slowly separated, m. p. 144°. On recrystallisation from aqueous alcohol, this *acetoxymercuri*-compound formed shining needles, m. p. 151—153° (Found: Hg, 44·0, 44·0; OMe, 6·7. $C_{11}H_{12}O_5SHg$ requires Hg, 44·1; OMe, 6·8%. The hydroxy-addition product $C_{10}H_{10}O_5SHg$ requires Hg, 45·3%). Hydrogen sulphide in aqueous solution gives mercuric sulphide; hydrochloric acid regenerates the sulphone; bromine and hydrogen bromide in cold glacial acetic quickly give thionaphthen sulphone dibromide, m. p. and mixed m. p. 164—165° (Lanfry, *loc. cit.*).

Alternative Preparations of Dihydrothionaphthen Sulphone.—Dihydrothionaphthen was prepared by the method of von Braun, Bayer, and Kayser (*loc. cit.*; see also Ferber, *ibid.*, 1929, **62**, 183; Bennett and Hafez, *J.*, 1941, 287). Phenylethyl chloride was nitrated (Barger, *J.*, 1909, **95**, 2194) and as much as possible of the solid *p*-nitro-compound removed. The liquid portion, containing also the *o*-derivative and traces of the *m*-compound (Holleman and Hoeflake, *Rec. Trav. chim.*, 1915, **34**, 261), was reduced (Ferber, *loc. cit.*), diazotised, and converted by the xanthate method and ring-closure into dihydrothionaphthen (the isomeric amines did not interfere). The crude product (C), b. p. 110—111°/18 mm. (v. Braun *et al.* give 104°/13 mm.), was twice fractionated giving (D) of b. p. 110—111°/18 mm. (Found: C, 71·3; H, 6·3; Calc. for C_8H_8S: C, 70·6; H, 5·9%). This with potassium permanganate gave a sulphone (B) of m. p. 92° (Found: C, 56·8; H, 4·9; S, 19·4. Calc. for $C_8H_8O_2S$: C, 57·1; H, 4·8; S, 19·0%). (D) was converted into the mercurichloride, m. p. 128° (Fricke and Spilker, *loc. cit.*), from which the dihydrothionaphthen was regenerated by dilute hydrochloric acid and converted into sulphone (E) as before, m. p. 92°. Fricke and Spilker (*loc. cit.*) give m. p. 91·5—92° (uncorr.); v. Braun *et al.*, and Bennett and Hafez found m. p. 98°. Preparations (A) (see above) and (B) were of entirely different origin, and (E) was obtained by a rigorous purification of (D). They all melted constantly at 92°.

Nitration of Dihydrothionaphthen and of its Sulphone.—Dihydrothionaphthen (0·5 g.) was added dropwise to cooled nitric acid (*d* 1·5), and the solution poured on ice. No solid separated, and the mixture was evaporated to a syrup. This partially solidified on being washed with sodium carbonate. When washed with methyl alcohol and recrystallised from aqueous alcohol, it melted at 142°. In admixture with thionaphthen sulphone (m. p. 142°) the m. p. was depressed. The quantity was too small for analysis but the compound probably consisted of mononitrodihydrothionaphthen sulphone. Dihydrothionaphthen sulphone (specimen B) was dissolved in nitric acid (*d* 1·5) and left for a short time at room temperature. Dilution with water gave colourless plates, m. p. 143°, on recrystallisation from

alcohol (Found: C, 44·9; H, 3·2; N, 6·7. $C_8H_7O_4NS$ requires C, 45·3; H, 3·3; N, 6·6%). The same *compound*, m. p. and mixed m. p. 143°, was obtained on nitration of specimen (A).

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328. *Amidines. Part X. Preparation of Amidines from Substituted Amides, a Sulphonyl Chloride, and an Amine.*

By P. OXLEY, D. A. PEAK, and W. F. SHORT.

Amidines are prepared by the reaction of amines with imidosulphonates, produced *in situ*, from *N*-monosubstituted amides and sulphonyl chlorides in presence of pyridine.

AMIDINES are obtained in good yield by carrying out the Beckmann rearrangement of ketoxime sulphonates in presence of ammonia or an amine, so that the intermediate imidosulphonate undergoes immediate ammonolysis (Part IX; this vol., p. 1514). If no ammonia or amine is present, the imidosulphonate undergoes further change with production of a sulphonic anhydride and an acylamidine, this transformation being retarded by pyridine. Since *N*-substituted amides are alkylated on nitrogen or on oxygen according to the experimental conditions (Lander, *J.*, 1903, **83**, 766; Grunfeld, *Bull. Soc. chim.*, 1936, **3**, 668), and there is evidence that imido-esters may be formed as intermediates in the production of diacylamines, $(R{\cdot}CO)_2NR'$, by the acylation of amides (Mumm, Hesse, and Volquartz, *Ber.*, 1915, **48**, 379; cf. Brunner, *Monatsh.*, 1915, **36**, 509), it seemed possible that *N*-substituted imidosulphonates would be formed by the action of sulphonyl chlorides on these amides and could be preserved in presence of pyridine long enough to yield amidines on subsequent addition of amines:

$$R{\cdot}CO{\cdot}NHR' \longrightarrow R{\cdot}C(:NR'){\cdot}O{\cdot}SO_2Ar \xrightarrow{R''{\cdot}NH_2} R{\cdot}C(:NR'){\cdot}\overset{\oplus}{N}H_2R''\}Ar\overset{\ominus}{S}O_3$$

We now report the preparation of a number of *NN'*-disubstituted and -trisubstituted amidines from anilides and other *N*-monosubstituted amides, including 2-ketohexamethyleneimine, by dissolving them in pyridine and adding successively a sulphonyl chloride and an amine. Attempts to prepare *N*-phenylbenzamidine from benzamide, benzenesulphonyl chloride, and aniline were unsuccessful since the imidosulphonate decomposed even at − 10° and the sole products were phenyl cyanide and phenylammonium benzenesulphonate.

EXPERIMENTAL.*

NN'-Diphenylformamidine.—Benzenesulphonyl chloride (18 g.; 1·1 mols.) was slowly added at − 10° to a solution of formanilide (11·1 g.) in dry pyridine (25 c.c.; 3·4 mols.) and, after a further 40 minutes' stirring at − 10°, aniline (10 g.; 1·2 mols.) was gradually added. The cooling bath was removed and when the mixture had warmed to room temperature it was heated on the steam-bath for ½ hour. The semi-solid product was diluted with benzene (50 c.c.) and cooled, and the crystals were filtered off and washed with benzene, giving *NN'*-diphenylformamidinium chloride, m. p. 250—252° (18·5 g., 86·5%). Recrystallisation from water (charcoal) afforded colourless prisms of the pure chloride, m. p. 252—254° (decomp.). Nef (*Annalen*, 1892, **270**, 297) records m. p. 255°. The amidine salt must be cautiously crystallised from water, otherwise complete hydrolysis occurs. The free base, liberated by means of ammonia, had m. p. 135°. Nef (*loc. cit.*) gives 138° as the m. p. of *NN'*-diphenylformamidine; Dains (*Ber.*, 1902, **35**, 2498) gives 143°.

NN'-Diphenylacetamidine.—A suspension of acetanilide (15 g.) in alcohol-free chloroform (50 c.c.) was added to a solution of benzenesulphonyl chloride (19·6 g.; 1 mol.) in pyridine (15 c.c.; 1·7 mols.) and alcohol-free chloroform (30 c.c.). The temperature rose from 20° to 27° and, after being stirred at room temperature for 3 hours, the red solution was added during 45 minutes at 22—25° to a mixture of aniline (10·5 g.; 1·0 mol.), pyridine (5 c.c.; 0·6 mol.), and chloroform (10 c.c.). The resulting solution was washed with aqueous sodium carbonate, dried ($MgSO_4$), evaporated to dryness in a vacuum, and the residue dissolved in cold alcohol (25 c.c.). The crystals which separated on adding water (25 c.c.) were collected, washed with aqueous alcohol (1 : 1), and dried, giving 15·2 g. of crude amidine, m. p. 115—123°. When the filtrate was acidified with dilute hydrochloric acid, acetanilide (2·5 g.) was recovered, and addition of ammonia to the acid filtrate gave a further precipitate of amidine, bringing the total yield to 69·5%. Recrystallisation from aqueous alcohol raised the m. p. of the amidine to 130—131° (see Part IX, *loc. cit.*) and the picrate had m. p. 168—169°. Von Pechmann (*Ber.*, 1897, **30**, 2792, 2879) records m. p. 169° for the picrate, and Oxley and Short (*J.*, 1947, 384) give m. p. 171°.

NN'-Diphenylbenzamidine.—There was no reaction below 50° when benzenesulphonyl chloride (9 g.; 1 mol.) was added to a solution of benzanilide (9·85 g.) in dry pyridine (12 c.c.; 3 mols.). The

* See also B.P. 577,478.

mixture was heated on the steam-bath for 90 minutes, and aniline (9 g.; 1·9 mols.) was then added to the hot solution and the mixture heated on the steam-bath for a further 90 minutes. The crystals which separated on cooling were washed with a little pyridine and consisted of *NN'*-diphenylbenzamidinium chloride, m. p. 300° (8·3 g., 54%). Sach and Bry (*Ber.*, 1901, **34**, 118) record m. p. 286° (decomp.) and Busch and Falco (*ibid.*, 1910, **43**, 2557) give m. p. 299° (decomp.). The free base had m. p. 146·5—147°, undepressed on admixture with an authentic specimen (Part I, *J.*, 1946, 147). Addition of water to the filtrate afforded recovered benzanilide, m. p. and mixed m. p. 160—163° (3·8 g., 39%), and an oil, which was probably the pyridine salt of benzenesulphonanilide, since this anilide, m. p. and mixed m. p. 107—108° (4·5 g., 39%), was obtained when it was shaken with dilute hydrochloric acid.

N-*Phenyl*-N'-*methylbenzamidine*.—Toluene-*p*-sulphonyl chloride (9·6 g.; 1 mol.) was added to a solution of benzmethylamide (6·8 g.) in dry pyridine (10 c.c.; 2·5 mols.), and, after this had been heated on the steam-bath for 2 hours, aniline (5 g.; 1·1 mols.) was added, after cooling slightly. The mixture was then heated on the steam-bath for a further 1½ hours, cooled, diluted with alcohol (50 c.c.), and filtered from an insoluble gum containing a few crystals. The filtrate was made alkaline with 5N-sodium hydroxide to liberate the crude amidine, m. p. 133—134° (6·35 g., 60%), which was collected and recrystallised from 50% aqueous alcohol, giving *N*-phenyl-*N'*-methylbenzamidine, m. p. 134·4—134·8°. Pyman (*J.*, 1923, **123**, 3366) records m. p. 135° (corr.).

N-*Methyl*-N'-n-*amylbenzamidine*.—Benzmethylamide (13·6 g.), pyridine (10 c.c.), benzene (50 c.c.), toluene-*p*-sulphonyl chloride (19·2 g.; 1 mol.), and *n*-amylamine (8·7 g.; 1 mol.) were brought into reaction as described in the last example, and the cold reaction product was poured into aqueous sodium hydroxide at 0°. The oil was collected (5·6 g.) and distilled under diminished pressure, and the fraction (5 g.), b. p. 125—128°/1·5 mm., was triturated with benzene–light petroleum and filtered. The solid consisted of unchanged benzmethylamide (3 g.), and the evaporated filtrate afforded a picrate, m. p. 108—109°, which with alkali gave *N*-methyl-*N'*-*n*-amylbenzamidine (2 g., 9·7%), m. p. 40°. There was no depression in m. p. when the amidine and its picrate were mixed with authentic specimens prepared as described in Part III (*J.*, 1947, 384).

2-*Anilohexamethyleneimine*.—The temperature rose to 28° when benzenesulphonyl chloride (9 g.; 1 mol.) was added to a stirred solution of 2-ketohexamethyleneimine (5·7 g.) in dry pyridine (5 c.c.; 1·2 mols.) and benzene (20 c.c.). After the mixture had been stirred for another hour, addition of aniline (5 g.; 1·1 mols.) raised the temperature to 53°. The red solution was cooled and stirred at room temperature for 1½ hours during which an oil separated. The product was diluted with water (25 c.c.) and the aqueous layer was separated and made alkaline with sodium hydroxide solution. The 2-anilohexamethyleneimine, m. p. 95—100° (4 g., 42%), was collected and recrystallised from benzene, giving the pure compound, m. p. and mixed m. p. 105° (Part IX, *loc. cit.*).

NNN'-*Trimethylacetamidine* (Experiment by Dr. P. T. Charlton).—Benzenesulphonyl chloride (17·65 g.; 1 mol.) was added during 15 minutes at 4° to a solution of acetomethylamide (7·3 g.) in a mixture of dry chloroform (80 c.c.) and pyridine (10 g.). The solution was stirred for 6 hours and a solution of dimethylamine (4·1 g.; 1 mol.) in chloroform was then added, stirring was continued for 12 hours, and the mixture was boiled under reflux for 2 hours. The dark solution was extracted with excess of dilute aqueous sodium hydroxide and the alkaline solution was extracted with chloroform. Evaporation of the chloroform solution afforded benzenesulphondimethylamide (7%), m. p. and mixed m. p. 47—48° (Found: N, 7·6. Calc. for $C_8H_{11}O_2NS$: N, 7·6%). The alkaline solution was acidified with hydrochloric acid, evaporated to dryness under diminished pressure, and the residue extracted with ethanol. The alcohol was removed and the residue recrystallised from acetone to give NNN'-*trimethylacetamidinium benzenesulphonate* as hygroscopic plates, m. p. 117—118° (Found: N, 10·7; S, 12·3. $C_{11}H_{15}O_3N_3S$ requires N, 10·85; S, 12·4%); yield, 8·5%. The *picrate*, prepared from the benzenesulphonate and saturated aqueous lithium picrate, separated from ethanol in yellow prisms, m. p. 96° (Found: N, 21·25. $C_{11}H_{15}O_7N_6$ requires N, 21·3%).

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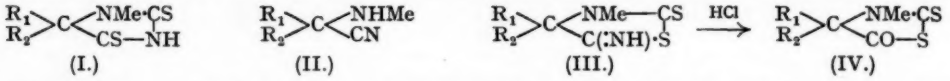
329. *The Reaction between Carbon Disulphide and Some α-Methylamino-nitriles.*

By H. C. Carrington.

The α-methylamino-nitriles derived from aliphatic or alicyclic ketones react with carbon disulphide to give 4 : 4-disubstituted 5-imino-3-methylthiazolidine-2-thiones, which are readily hydrolysed by acid to the corresponding thiazolidone-2-thiones. A member of the latter series, 3 : 4 : 4-trimethylthiazolid-5-one-2-thione, has also been synthesised by treating an alkaline solution of α-methylamino*iso*butyric acid with carbon disulphide and acidifying the product.

During work on the synthesis of 5 : 5-disubstituted 1-methyl-2 : 4-dithiohydantoins (I) it appeared desirable to investigate the possibility of preparing these compounds by the action of carbon disulphide on the α-methylamino-nitriles (II) derived from ketones. Bucherer and Lieb (*J. pr. Chem.*, 1934, **141**, 5), in a study of the formation of hydantoins from ketones, found that 5 : 5-disubstituted 1-methylhydantoins were not produced when ketone cyanohydrins were treated with methylamine and carbon dioxide, but Jacobson, in a patent describing the formation

of 2 : 4-dithiohydantoins by the action of carbon disulphide on α-amino-nitriles (U.S.P. 2,143,816, 1939), stated that 1 : 5 : 5-trimethyl-2 : 4-dithiohydantoin (I; $R_1 = R_2 = Me$) could be prepared



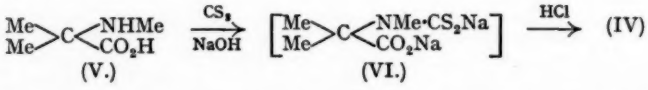
(I.) (II.) (III.) (IV.)

in this way from α-methylamino*iso*butyronitrile (II; $R_1 = R_2 = Me$) as a colourless crystalline substance, m. p. 83·5—84·5°.

1 : 5 : 5-Trimethylhydantoin has been prepared by Biltz and Slotta (*J. pr. Chem.*, 1926, **113**, 233; see also Stuckey, *J.*, 1947, 331) by the action of cyanate on α-methylamino*iso*butyronitrile and treatment of the product with acid. On reaction with phosphorus pentasulphide and " liver of sulphur " in boiling xylene it gave a yellow crystalline compound, m. p. 146—147°, which was soluble in cold dilute aqueous sodium hydroxide, was reprecipitated by acid, and was, in fact, authentic 1 : 5 : 5-*trimethyl*-2 : 4-*dithiohydantoin*.

A reinvestigation of the reaction between α-methylamino*iso*butyronitrile and carbon disulphide showed the main product to be 5-*imino*-3 : 4 : 4-*trimethylthiazolidine*-2-*thione* (III; $R_1 = R_2 = Me$), a bright yellow crystalline substance, m. p. 115°, isomeric with 1 : 5 : 5-trimethyl-2 : 4-dithiohydantoin, but with quite different properties. It was insoluble in cold dilute aqueous sodium hydroxide, but readily soluble in cold dilute hydrochloric acid. From the solution in acid there rapidly separated 3 : 4 : 4-*trimethylthiazolid*-5-*one*-2-*thione* (IV; $R_1 = R_2 = Me$), a colourless crystalline compound, m. p. 85°, in which the original 5-imino-group was replaced by oxygen.

The constitution of the latter substance was confirmed by an alternative synthesis. An alkaline solution of α-methylamino*iso*butyric acid (V), shaken for several days with carbon disulphide, gave on acidifying, in small yield, 3 : 4 : 4-trimethylthiazolid-5-one-2-thione identical with that described above. This synthesis probably proceeds through the salt of the dithiocarbamic acid (VI).



(V.) (VI.) (IV)

It seems probable that the product obtained by Jacobson was 3 : 4 : 4-trimethylthiazolid-5-one-2-thione, although no intervention of acid was mentioned in the description of his process. The melting point and appearance are in agreement with this supposition. A nitrogen analysis would have clarified the situation, but none was reported, and the other analytical figures would not disclose the replacement of an imino-group by an oxygen atom.

α-Methylamino-α-methylbutyronitrile (II; $R_1 = Me$, $R_2 = Et$) with carbon disulphide similarly gave the yellow crystalline 5-*imino*-3 : 4-*dimethyl*-4-*ethylthiazolidine*-2-*thione* (III; $R_1 = Me$, $R_2 = Et$), which was readily hydrolysed by dilute acid to 3 : 4-*dimethyl*-4-*ethyl*-*thiazolid*-5-*one*-2-*thione* (IV; $R_1 = Me$, $R_2 = Et$).

The yellow 5-imino-compounds were readily converted by warm aqueous alcoholic sodium hydroxide into isomeric colourless forms, which with dilute acid readily gave the same thiazolid-5-ones as were obtained from the yellow forms. The relationship between the two forms of the 5-imino-compounds has not yet been elucidated, and no method of reconverting the colourless forms into the yellow ones has been discovered. In view of the facility with which both forms are hydrolysed to the same thiazolid-5-ones, it is difficult to postulate any structural difference between them. The yellow 5-imino-compounds were very readily acetylated by acetic anhydride in the cold, and the *acetyl* derivatives, on treatment with aqueous-alcoholic sodium hydroxide, were deacetylated to the colourless forms.

The 5-imino-group of 5-imino-3 : 4 : 4-trimethylthiazolidine-2-thione was readily replaced by other basic groups. For instance, on warming with aqueous 2-aminoethyl alcohol, ammonia was evolved, and the corresponding 5-(2′-*hydroxyethylimino*)-3 : 4 : 4-*trimethylthiazolidine*-2-*thione* was produced. This substance, like the parent 5-imino-compound, was readily soluble in dilute acid, and hydrolysed to the corresponding thiazolid-5-one on warming.

When the α-methylamino-nitriles from cyclic ketones reacted with carbon disulphide, the corresponding 5-iminothiazolidine-2-thiones were obtained as colourless crystalline solids. For example, 1-methylamino-1-cyano*cyclo*hexane (II; $R_1R_2 = CH_2{<}[CH_2]_4{>}$) gave 5-*imino*-4 : 4-cyclo*pentamethylene*-3-*methylthiazolidine*-2-*thione* (III; $R_1R_2 = CH_2{<}[CH_2]_4{>}$), and 1-methylamino-1-cyano-4-methyl*cyclo*hexane (II; $R_1R_2 = CH_3{\cdot}CH{<}[CH_2]_4{>}$) gave 5-*imino*-4 : 4-(3′-*methyl*cyclo*pentamethylene*)-3-*methylthiazolidine*-2-*thione* (III; $R_1R_2 = CH_3{\cdot}CH{<}[CH_2]_4{>}$).

No yellow forms of these substances were observed. They were readily hydrolysed by dilute acid to the corresponding 4 : 4-cyclo*pentamethylene*-3-*methyl*- (IV; $R_1R_2 = CH_2<[CH_2]_4>$) and 4 : 4-(3′-*methyl*cyclo*pentamethylene*)-3-*methyl*-*thiazolid*-5-*one*-2-*thione* (IV; $R_1R_2 = CH_3{\cdot}CH<[CH_2]_4>$), respectively.

Experimental.

Reaction of α-Methylamino-nitriles with Carbon Disulphide.—α-Methylamino*iso*butyronitrile (60 g.) and carbon disulphide (80 g.) were heated in a water-bath at 50° under reflux for 24 hours. The excess of carbon disulphide was removed under diminished pressure and the residual oil crystallised on standing. Benzene (50 c.c.) was added, and the crystalline solid was separated, washed with benzene, and recrystallised from aqueous methanol to give 5-*imino*-3 : 4 : 4-*trimethylthiazolidine*-2-*thione* as large yellow plates, m. p. 115—116°, in 48% yield (Found : C, 41·2; H, 5·5; N, 16·4; S, 36·9. $C_6H_{10}N_2S_2$ requires C, 41·35; H, 5·75, N, 16·1; S, 36·8%). In a similar manner α-methylamino-α-methylbutyronitrile gave in 40% yield the yellow crystalline 5-*imino*-3 : 4-*dimethyl*-4-*ethylthiazolidine*-2-*thione*, m. p. 117° (Found : N, 14·5; S, 34·3. $C_7H_{12}N_2S_2$ requires N, 14·9; S, 34·1%). 1-Methylamino-1-cyano*cyclo*hexane gave in 30% yield the colourless crystalline 5-*imino*-4 : 4-cyclo*pentamethylene*-3-*methylthiazolidine*-2-*thione*, m. p. 125° (Found : N, 13·1; S, 29·9. $C_9H_{14}N_2S_2$ requires N, 13·1; S, 29·9%). 1-Methylamino-1-cyano-4-methyl*cyclo*hexane gave in 30% yield the colourless crystalline 5-*imino*-4 : 4-(3′-*methyl*cyclo*pentamethylene*)-3-*methylthiazolidine*-2-*thione*, m. p. 146—147° (Found : N, 12·0; S, 27·8. $C_{10}H_{16}N_2S_2$ requires N, 12·3; S, 28·1%).

Acid Hydrolysis of the Imino-compounds.—The imino-group of the 3 : 4 : 4-trisubstituted 5-iminothiazolidine-2-thiones was readily replaced by oxygen by warming with dilute aqueous or aqueous-alcoholic hydrochloric acid. The trimethyl compound dissolved readily in cold 7% hydrochloric acid and the product of hydrolysis separated on standing at room temperature for a few minutes. The 3 : 4-dimethyl-4-ethyl homologue was soluble in cold dilute hydrochloric acid, and the solution was heated for 10 minutes on the steam-bath to complete the hydrolysis. The two compounds with cyclic substituents in the 4-position were not readily soluble in dilute aqueous hydrochloric acid. They were dissolved in aqueous-alcoholic hydrochloric acid, the hydrolysis was completed by heating on the steam-bath for ½ hour, and the colourless crystalline product separated on cooling. In all cases the yield was nearly quantitative, and the products were purified by crystallisation from aqueous methanol. There were thus obtained 3 : 4 : 4-*trimethyl*-, m. p. 85° (Found : N, 7·8; S, 36·8. $C_6H_9ONS_2$ requires N, 8·0; S, 36·6%); 3 : 4-*dimethyl*-4-*ethyl*-, m. p. 76° (Found : N, 7·0; S, 33·5. $C_7H_{11}ONS_2$ requires N, 7·4; S, 33·8%), 4 : 4-cyclo*pentamethylene*-3-*methyl*-, m. p. 85° (Found : N, 6·8; S, 29·7. $C_9H_{13}ONS_2$ requires N, 6·5; S, 29·8%), and 4 : 4-(3′-*methyl*cyclo*pentamethylene*)-3-*methylthiazolid*-5-*one*-2-*thione*, m. p. 110° (Found : N, 6·0; S, 28·4. $C_{10}H_{15}ONS_2$ requires N, 6·1; S, 28·0%).

The Two Forms of 3 : 4 : 4-Trisubstituted 5-Iminothiazolidine-2-thiones.—The yellow form of 5-imino-3 : 4 : 4-trimethylthiazolidine-2-thione (m. p. 115°), on warming with aqueous-alcoholic sodium hydroxide and cooling, gave colourless needles, which after recrystallising from aqueous methanol had m. p. 58°, rising to 70° with loss of solvent on drying in a vacuum (Found, on dried material : N, 15·9; S, 37·0. $C_6H_{10}N_2S_2$ requires N, 16·1; S, 36·8%). On warming for a few minutes with dilute hydrochloric acid this colourless *form* was hydrolysed to 3 : 4 : 4-trimethylthiazolid-5-one-2-thione, m. p. 85°, identical with that similarly obtained from the yellow form. The yellow form of 5-imino-3 : 4-dimethyl-4-ethylthiazolidine-2-thione (m. p. 117°) on treatment with warm aqueous-alcoholic sodium hydroxide was converted into a colourless *form*, crystallising from aqueous methanol with m. p. 85° (Found : N, 14·65; S, 34·1. $C_7H_{12}N_2S_2$ requires N, 14·9; S, 34·1%). With dilute hydrochloric acid this gave 3 : 4-dimethyl-4-ethylthiazolid-5-one-2-thione, m. p. 76°, identical with that obtained from the yellow form.

The 5-imino-3-methylthiazolidine-2-thiones with cyclic substituents in the 4-position, which were originally isolated in colourless form, were unchanged on treatment with warm aqueous-alcoholic sodium hydroxide, and no yellow forms of these compounds have been obtained.

Derivatives of 3 : 4 : 4-Trisubstituted 5-Iminothiazolidine-2-thiones.—(a) *Acetyl derivatives.* 5-Imino-3 : 4 : 4-trimethylthiazolidine-2-thione (yellow form, m. p. 115°, 2 g.) was mixed with acetic anhydride (1 c.c.); a vigorous reaction took place, and after being stirred with water the solid product was filtered off and crystallised from aqueous methanol to give the *acetyl* derivative as colourless crystals (1·5 g.), m. p. 147—148° (Found : C, 44·5; H, 5·6. $C_8H_{12}ON_2S_2$ requires C, 44·8; H, 5·7%). The acetyl derivative (1 g.) was warmed with 4% aqueous-alcoholic sodium hydroxide (8 c.c.) on the steam-bath for 10 minutes. On cooling there separated the colourless form of 5-imino-3 : 4 : 4-trimethylthiazolidine-2-thione, m. p. 58°, rising to 70° on drying in a vacuum, identical with that described above. In a similar way 5-imino-3 : 4-dimethyl-4-ethylthiazolidine-2-thione (yellow form, m. p. 117°) gave a colourless *acetyl* derivative, m. p. 112° (Found : N, 12·1; S, 28·0. $C_9H_{14}ON_2S_2$ requires N, 12·2; S, 27·8%). Deacetylation with aqueous-alcoholic sodium hydroxide gave the colourless form of the imino-compound, m. p. 85°, identical with that described above.

(b) 2-*Hydroxyethyl derivative.* 5-Imino-3 : 4 : 4-trimethylthiazolidine-2-thione (yellow form, m. p. 115°, 2 g.) was heated on the steam-bath for 10 minutes with 2-aminoethanol (4 c.c.) and water (4 c.c.). Ammonia was evolved, with traces of hydrogen sulphide. On cooling, a white crystalline solid separated. After crystallising from aqueous methanol 5-(2′-*hydroxyethylimino*)-3 : 4 : 4-*trimethylthiazolidine*-2-*thione* had m. p. 100° (Found : C, 43·85; H, 6·3. $C_8H_{14}ON_2S_2$ requires C, 44·0; H, 6·4%). This compound was readily soluble in cold dilute hydrochloric acid, and on warming the solution there was produced 3 : 4 : 4-trimethylthiazolid-5-one-2-thione, m. p. 85°, identical with that previously described.

*Reaction of α-Methylamino*iso*butyric Acid with Carbon Disulphide.*—α-Methylamino*iso*butyric acid (1 g.) in 8% aqueous sodium hydroxide (10 c.c.) was shaken with carbon disulphide (2 c.c.) for 3 days. The unchanged carbon disulphide was separated, and the yellow solution acidified with hydrochloric acid. The colourless solid was separated (0·1 g.), and after crystallisation from aqueous methanol it had m. p. 85°, and was identical with the 3 : 4 : 4-trimethylthiazolid-5-one-2-thione described above.

1 : 5 : 5-*Trimethyl*-2 : 4-*dithiohydantoin*.—A suspension of 1 : 5 : 5-trimethylhydantoin (5 g.), finely ground phosphorus pentasulphide (5 g.), and liver of sulphur (6 g.) in dry xylene (100 c.c.) was stirred and boiled under reflux for 18 hours. The hot mixture was filtered, and the product, which separated from the filtrate on cooling, was crystallised from aqueous methanol to give 1 : 5 : 5-*trimethyl*-2 : 4-*dithiohydantoin* as long pale yellow plates (1 g.), m. p. 146—147° (Found : N, 15·9; S, 37·2. $C_6H_{10}N_2S_2$ requires N, 16·1; S, 36·8%.

The author wishes to thank Mr. E. S. Morton, who carried out the microanalyses.

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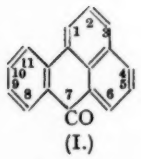
330. *Kationoid Reactivity of Aromatic Compounds. Part VII. The Composition of the Di*mesobenzanthronyl *formed by the Action of Alkaline Condensing Agents on* meso*Benzanthrone.*

By W. BRADLEY and G. V. JADHAV.

The action of alcoholic potassium hydroxide or sodium anilide on *meso*benzanthrone yields 4 : 4′-di*meso*benzanthronyl with less than 2·5% of 6 : 6′-*di*mesobenzanthronyl. The preparation of the latter from 4-hydroxy*meso*benzanthrone is described. Neither 4-methyl- nor 4-*fluoro*-meso*benzanthrone* shows evidence of coupling to a di*meso*benzanthronyl through the 6-position on treatment with alkaline condensing agents. The mode of formation of 4 : 4′-di-*meso*benzanthronyl from *meso*benzanthrone is discussed.

ALTHOUGH *meso*benzanthrone (I) reacts with sodamide–piperidine to form 4-piperidino*meso*-benzanthrone (Bradley, *J.*, 1937, 1091) and with alkyl- or aryl-magnesium iodides to yield 6-alkyl(or aryl)*meso*benzanthrones (Allen and Overbaugh, *J. Amer. Chem. Soc.*, 1935, **57**, 740; Charrier and Ghigi, *Ber.*, 1936, **69**, 2211), yet fused potassium hydroxide in the presence of an oxidant yields both 4- and 6-hydroxy*meso*benzanthrones (Bradley and Jadhav, *J.*, 1937, 1791). Following Badische, B.P. 203,533, Lüttringhaus and Neresheimer (*Annalen*, 1929, **473**, 259) showed that alcoholic potassium hydroxide or sodium anilide transforms *meso*benzanthrone into 4 : 4′-di*meso*benzanthronyl (II) but no reference was made to the possible occurrence of an



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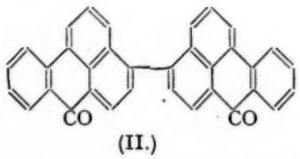

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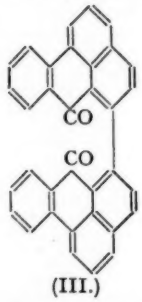

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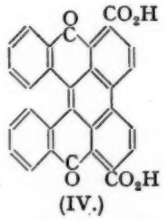


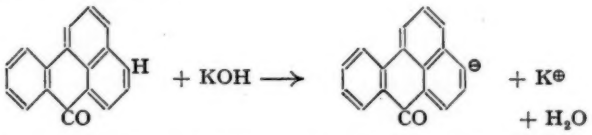
(IV.)

isomer of (II) in the crude product. The present experiments were carried out to ascertain whether 6 : 6′-*di*mesobenzanthronyl (III) is also present. 6-Hydroxy*meso*benzanthrone, prepared by the action of aluminium chloride on 1-benzoyl-2-naphthol, was heated with aqueous ammonia to give 6-amino*meso*benzanthrone. From this was obtained 6-chloro-, 6-*bromo*- and 6-*iodo*-meso*benzanthrone* by the Sandmeyer reactions. 6 : 6′-Di*meso*benzanthronyl was prepared by heating 6-chloro*meso*benzanthrone with copper powder. 6 : 6′-Di*meso*benzanthronyl gives a green coloration with acetone in the presence of solid potassium hydroxide. Under the same conditions both *meso*benzanthrone and 4 : 4′-di*meso*benzanthronyl give violet colours, and the difference could have been used to detect the presence of the 6 : 6′- in the 4 : 4′-isomer had not the rate of development of the green colour been retarded and masked by 4 : 4′-di*meso*-benzanthronyl. 6 : 6′-Di*meso*benzanthronyl is easily oxidised by means of chromium trioxide and the resulting 1 : 1′-dianthraquinonyl-4 : 4′-dicarboxylic acid gives a green colour with copper and sulphuric acid, doubtless because of the formation of meso*benzodianthrone*-6 : 6′-*dicarboxylic acid* (IV).

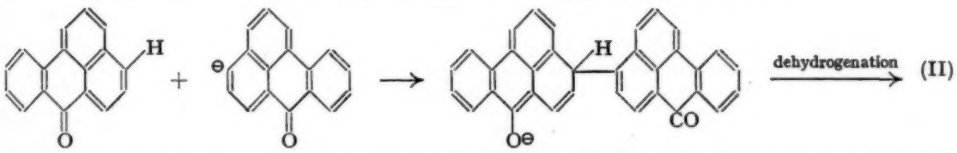
4 : 4′-Di*meso*benzanthronyl, oxidised similarly, affords 2 : 2′-dianthraquinonyl-1 : 1′-dicarboxylic acid. This does not give any green colour when treated with copper and sulphuric acid, a

reaction which is characteristic of 1 : 1′-dianthraquinonyls (Scholl and Mansfield, *Ber.*, 1910, **43**, 1734). In control experiments it was found that mixtures of 4 : 4′- and 6 : 6′-di*meso*benzanthronyls containing at least 2·5% of the latter, when oxidised and the resulting acids treated with copper and sulphuric acid, gave a green coloration. An identical result was obtained when a portion of the 4 : 4′-isomer was replaced by *meso*benzanthrone. The test was negative when applied to the acids obtained by oxidising the crude di*meso*benzanthronyl prepared from *meso*benzanthrone and either alcoholic potassium hydroxide or sodium anilide, an indication that crude di*meso*benzanthronyl does not contain more than 2·5% of the 6 : 6′-isomer.

Mode of Formation of 4 : 4′-*Dimesobenzanthronyl.*—The conversion of *meso*benzanthrone into 4 : 4′-di*meso*benzanthronyl by means of an alkaline condensing agent suggests that a typical substitution into *meso*benzanthrone is involved, the substituting agent being an anion, and the point of substitution a carbon atom situated *para* to the carbonyl group (cf. Part VI, this vol., p. 1175). The constitution of the product indicates that the anion is derived from *meso*benzanthrone by loss of a proton;



and the formation of 4 : 4′-di*meso*benzanthronyl can be represented as follows:

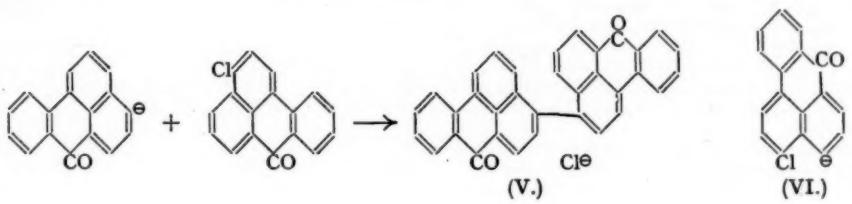


The dissociation of hydrogen as proton from its attachment to carbon is familiar in such examples as acetylene, indene, fluorene, and hydrogen cyanide, and the process is facilitated by the attachment to carbon of electron-seeking groups (phenylnitromethane, dibenzenesulphonylmethane).

Although the self-coupling of *meso*benzanthrone is the best known example of its type, several other carbonyl compounds yield analogous products when they are heated with alkalis. 1 : 9-Pyrazoleanthrone yields pyrazoleanthrone-yellow, the 1 : 9-pyrazoleanthrone analogue of 4 : 4′-di*meso*benzanthronyl (*Chem.-Ztg.*, Fortschrittsberichte, 1929, p. 56), 5(*N*) : 6-pyridino-*meso*benzanthrone yields cyananthrone (Bally, *Ber.*, 1905, **38**, 196), and anthracene-1 : 9-dicarboxyimide yields aceanthrene-green (Kardos, *Ber.*, 1913, **46**, 2086; Houben, "Das Anthracen und die Anthrachinone", p. 771, Leipzig, 1929). It is very probable that these reactions, too, involve the same principle as the formation of 4 : 4′-di*meso*benzanthronyl from *meso*benzanthrone.

The occurrence of an anion derived from *meso*benzanthrone by loss of a proton provides a means of explaining a number of reactions related to the self-condensation of *meso*benzanthrone. Lüttringhaus and Neresheimer found that replacing a hydrogen in the 9-position of *meso*benzanthrone did not inhibit the formation of a 9 : 9′-disubstituted 4 : 4′-di*meso*benzanthronyl, and further that (following Scottish Dyes, B.P. 251,313) the previous linking of two *meso*benzanthrone molecules through the 3-positions did not inhibit the subsequent union through the 4-positions also, to give violanthrone, by the action of alkaline condensing agents.

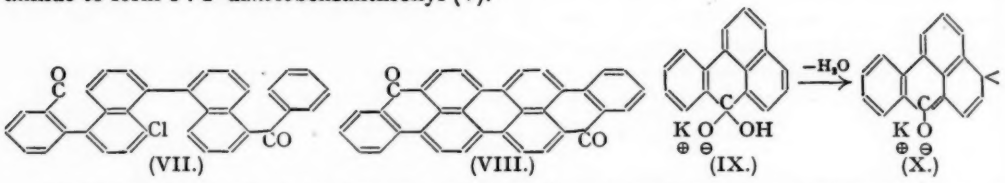
The anion of *meso*benzanthrone replaces chlorine attached to the 4- and the 3-position of *meso*benzanthrone more easily than it substitutes into the nucleus. Even at − 5° an equimolecular mixture of *meso*benzanthrone and 3-chloro*meso*benzanthrone forms 3 : 4′-di*meso*benzanthronyl (V) in the presence of sodium anilide. This reaction may be represented as follows:



When the halogen compound is 4-chloro*meso*benzanthrone the product is analogously 4 : 4′-di*meso*benzanthronyl. The action of alcoholic potassium hydroxide on 3-chloro*meso*-benzanthrone yields *iso*violanthrone (VIII). In this reaction 3-chloro-3′ : 4-di*meso*benzanthonyl (VII) is an intermediate product.

In this reaction it is apparent that 3-chloro*meso*benzanthrone yields the anion (VI) which is able to replace chlorine in another molecule of 3-chloro*meso*benzanthrone giving (VII). Repetition of the processes of proton liberation and halogen replacement then leads to *iso*violanthrone (VIII).

Similarly *meso*benzanthrone reacts with 3-fluoro*meso*benzanthrone in the presence of sodium anilide to form 3 : 4′-di*meso*benzanthronyl (V).



(VII.) (VIII.) (IX.) (X.)

In an earlier theory of the formation of 4 : 4′-di*meso*benzanthronyl and violanthrone from *meso*benzanthrone (Schwenk, *Chem.-Ztg.*, 1928, **52**, 62) it was suggested that the action of alkali hydroxides on *meso*benzanthrone was first to form the adduct (IX) which lost water to give the ion-radical (X) and finally 4 : 4′-di*meso*benzanthronyl by polymerisation and oxidation. This view of the course of the reaction is intrinsically improbable, and it does not accommodate the analogous reactions of *meso*benzanthrone with halogenated *meso*benzanthrones in which the halogen is eliminated as an anion.

Experimental.

6-*Hydroxy*meso*benzanthrone*.—An intimate mixture of 1-benzoyl-2-naphthol (10 g.) and anhydrous aluminium chloride (25 g.) was added in small successive portions during 45 minutes to a beaker maintained at 140—150°. The melt was stirred throughout. The cooled product was extracted with boiling concentrated hydrochloric acid, then with water and finally with hot glacial acetic acid. It crystallised from the last (charcoal) in the form of yellow needles, m. p. 177—178°.

6-*Amino*meso*benzanthrone*.—A suspension of 6-hydroxy*meso*benzanthrone (8 g.) in aqueous ammonia (*d* 0·88; 160 c.c.) was heated for six hours at 210—220° (inner temperature) in an oil-jacketed autoclave. On cooling, water was added and the undissolved material collected. Crystallisation from alcohol gave golden-yellow prisms of 6-amino*meso*benzanthrone, m. p. 186—187° (Found: C, 82·9; H, 4·4; N, 5·6. Calc. for $C_{17}H_{11}ON$: C, 83·2; H, 4·5; N, 5·7%) (yield, 6 g.), identical with the amino*meso*benzanthrone obtained by reaction of *meso*benzanthrone with sodamide in the presence of oxygen (Part VI, *loc. cit.*).

6-*Acetamido*meso*benzanthrone*, obtained by boiling 1 g. of 6-amino*meso*benzanthrone with 5 c.c. of acetic anhydride for an hour, crystallised from glacial acetic acid in brownish-yellow needles, m. p. 224—225° (Found: C, 79·5; H, 4·6; N, 5·1. $C_{19}H_{13}O_2N$ requires C, 79·4; H, 4·6; N, 4·9%).

6-*Benzamido*meso*benzanthrone*, prepared from 1 g. of 6-amino*meso*benzanthrone and 2 g. of benzoyl chloride by boiling the reactants for 30 minutes in pyridine solution, crystallised from glacial acetic acid in brownish-yellow needles, m. p. 204—205° (Found: C, 82·5; H, 4·4; N, 4·1. $C_{24}H_{15}O_2N$ requires C, 82·5; H, 4·3; N, 4·0%).

6-*Chloro*meso*benzanthrone*.—A solution of 6-amino*meso*benzanthrone (15 g.) in concentrated sulphuric acid (55 c.c.) was added gradually to a stirred solution of sodium nitrite (6 g.) in concentrated sulphuric acid (40 c.c.), the temperature being kept below 10°. Stirring was continued for an hour after the addition of the 6-amino*meso*benzanthrone was complete and then the product was poured over crushed ice. A brick-red solid separated. This was collected and washed with water. It was much more soluble in water than in dilute sulphuric acid. The red solid, made into a paste with 75 c.c. of concentrated hydrochloric acid, was added to a well-stirred solution prepared by dissolving 15 g. of cuprous chloride in 225 c.c. of 12% hydrochloric acid. Stirring was continued at room temperature until evolution of nitrogen ceased. The yellow undissolved solid was collected, washed, and crystallised from hot glacial acetic acid. Recrystallisation from alcohol gave yellow granules, m. p. 146—147° (Found: C, 77·1; H, 3·6; Cl, 13·3. Calc. for $C_{17}H_9OCl$: C, 77·1; H, 3·4; Cl, 13·4%). A product, m. p. 152°, considered to be 6-chloro*meso*benzanthrone was described by I.G. in B.P. 301,197 (1927).

The product (2 g.) obtained by the present preparation was oxidised by adding gradually a solution of 4·5 g. of chromium trioxide in 10 c.c. of 50% acetic acid. Carbon dioxide was evolved freely, and when the reaction was complete, water was added and the separated solid collected. After being washed, dissolved in dilute aqueous ammonia, and reprecipitated by addition of acid, it was crystallised from glacial acetic acid. The product, 4-chloroanthraquinone-1-carboxylic acid, melted at 229—230° (Found: C, 62·5; H, 2·5; Cl, 12·1. Calc. for $C_{15}H_7O_4Cl$: C, 62·8; H, 2·5; Cl, 12·4%). [Ullmann, D.R.-P. 243,788, Frdl., **10**, 598 (1909), records m. p. 229°; Heller and Shülke, *Ber.*, 1908, **41**, 3636, give m. p. 228—229°; Ullmann and Miriajeff, *Annalen*, 1912, **388**, 217, m. p. 229°; Stanley and Adams, *J. Amer. Chem. Soc.*, 1931, **53**, 2364, m. p. 220—224°; Heilbron, Heslop, and Irving, *J.*, 1936, 781, m. p. 231—232°.]

6-*Bromo*meso*benzanthrone*.—A solution of 6-amino*meso*benzanthrone (2·5 g.) in concentrated sulphuric acid (15 c.c.) was added at 5—10° to a solution of sodium nitrite (1 g.) in concentrated sulphuric acid (18 c.c.). The mixture was stirred for an hour and then poured on crushed ice. The precipitated

diazonium sulphate was collected, washed with a small volume of water, then made into a paste with water and added to a solution of cuprous bromide which had been prepared as follows: Copper powder (1 g.) and sodium bromide (8 g.) were added to a solution of copper sulphate (5 g.) in water (40 c.c.), and the suspension boiled until the solution was colourless. The suspension of the diazonium sulphate in the cuprous bromide solution was stirred at the room temperature until evolution of nitrogen ceased. The almost black solid product was collected, washed with water, dried, and then extracted by means of glacial acetic acid. The filtered extracts were combined, concentrated, and finally mixed with water. The *bromo*-compound separated as a yellow solid which crystallised from alcohol in needles, m. p. 144—145° (Found: C, 66·1; H, 2·9; Br, 25·6. $C_{17}H_9OBr$ requires C, 66·0; H, 2·9; Br, 25·9%).

4-*Bromoanthraquinone*-1-*carboxylic Acid*.—A solution of chromium trioxide (3 g.) in 50% acetic acid (8 c.c.) was added slowly to a hot solution of 6-bromo*meso*benzanthrone (1·5 g.) in glacial acetic acid (15 c.c.). Carbon dioxide was evolved and after the vigorous reaction had moderated the solution was boiled for three hours and then added to water. The solid which separated was collected, washed with water, and extracted by means of hot dilute ammonia. The filtered, reddish-yellow ammoniacal solution gave a yellowish solid when acidified, and this, after several recrystallisations from glacial acetic acid, had m. p. 250—251° (Found: C, 54·8; H, 2·3; Br, 24·4. $C_{15}H_7O_4Br$ requires C, 54·4; H, 2·1; Br, 24·1%).

6-*Iodo*meso*benzanthrone*.—The diazonium sulphate from 5 g. of 6-amino*meso*benzanthrone was made into a paste with water and stirred with a solution of 30 g. of sodium iodide in 200 c.c. of water. Nitrogen was evolved. The black *iodo*-compound, washed, dried, and crystallised several times from methanol, afforded small yellow needles that sintered at 125° and melted completely at 133° (Found: C, 57·3; H, 2·6; I, 35·3. $C_{17}H_9OI$ requires C, 57·3; H, 2·5; I, 35·7%).

6-N-*Methylanilino*meso*benzanthrone*, prepared by heating 6-chloro*meso*benzanthrone with an excess of monomethylaniline at the boiling point for four hours, crystallised from alcohol in yellow needles, m. p. 155—156° (Found: C, 86·1; H, 4·8; N, 4·6. $C_{24}H_{17}ON$ requires C, 86·0; H, 5·1; N, 4·2%).

6 : 6′-*Di*meso*benzanthronyl*.—A solution of 6-chloro*meso*benzanthrone (10 g.) in nitrobenzene (70 c.c.) was heated under reflux with copper dust (10 g.). The reaction proceeded smoothly for two hours but at this stage the separating solid caused bumping. After two hours longer an additional quantity of nitrobenzene (20 c.c.) was added, the suspension filtered, and the residue extracted with more nitrobenzene. On cooling, a yellow solid separated from the nitrobenzene solutions. It was insoluble in the common organic solvents. It did not melt below 355°. It was purified by extracting it with a large volume of acetone and then repeatedly crystallising the insoluble part from nitrobenzene. In this way it was obtained in the form of silky yellow needles, m. p. > 360° (Found: C, 88·3; H, 4·4. $C_{34}H_{18}O_2$ requires C, 89·1; H, 4·0%). 6 : 6′-*Di*meso*benzanthronyl* dissolved in concentrated sulphuric acid with a red colour. It was unaffected by stirring with aluminium powder and concentrated sulphuric acid at 30—40°, by warming with sodium amalgam and pyridine containing a small proportion of water, or by heating with amalgamated zinc and concentrated hydrochloric acid.

Reaction of 6 : 6′-*Di*meso*benzanthronyl with Sodium and Amyl Alcohol*.—Sodium (6 g.) was added to a boiling suspension of 6 : 6′-di*meso*benzanthronyl (2 g.) in amyl alcohol (60 c.c.). The colour changed from yellow to green. Soon after all of the metal had reacted, the colour began to change to reddish-brown. At this stage the product was cooled, mixed with water, and acidified with hydrochloric acid. The amyl alcohol was removed by steam-distillation, leaving a brown oil which solidified. The solid was collected, washed, and dried. It was soluble in alcohol, ether, benzene, or chloroform, but it could not be crystallised. It was precipitated from benzene solution on adding light petroleum, and several repetitions of this process gave a solid which melted in the range 205—220° (Found: C, 83·5; H, 6·0. $C_{44}H_{42}O_4$ requires C, 83·2; H, 6·7%).

Reaction of 6 : 6′-*Di*meso*benzanthronyl with Compounds containing Reactive Methylene Groups*.—A 0·1% solution of 6 : 6′-di*meso*benzanthronyl in pyridine (5 c.c.) with acetone (1 g.) and 1 g. of solid potassium hydroxide in the form of pellets changed in colour from yellow to green in the course of a few minutes. After two hours the solution acquired a violet tinge. No colour change was observed when the following compounds were used in the place of acetone; methyl ethyl ketone, diethyl ketone, acetophenone, propiophenone, butyrophenone, *cyclo*hexanone, diethyl malonate, ethyl acetoacetate, acetonitrile, propionitrile, benzyl cyanide, ethyl cyanoacetate. *meso*Benzanthrone gave a reddish colour becoming violet with acetone, and greenish, slowly fading, with acetophenone. 4 : 4′-Di*meso*benzanthronyl gave a green colour with acetophenone and a reddish colour changing to violet with acetone. The green colour produced by the interaction of 6 : 6′-di*meso*benzanthronyl and acetone does not appear so quickly if a small proportion of 4 : 4′-di*meso*benzanthronyl is present, and the green colour is soon masked by the violet colour due to the 4 : 4′-isomer.

1 : 1′-*Dianthraquinonyl*-4 : 4′-*dicarboxylic Acid*.—To a warm suspension of 6 : 6′-di*meso*benzanthonyl (1 g.) in glacial acetic acid (20 c.c.) was added gradually to a solution of chromium trioxide (2·5 g.) in 50% acetic acid (5 c.c.). Carbon dioxide was liberated vigorously, and when the initial reaction had ceased the product was boiled for five hours, then mixed with water and again heated to boiling. The solid which separated was collected, washed, and extracted by means of hot dilute sodium hydroxide solution. The filtered alkaline solution gave a yellow precipitate when acidified. The precipitated acid was almost insoluble in the common solvents. After drying it dissolved sparingly in pyridine and from the solution the *monopyridinium* salt crystallised in cream-coloured needles, m. p. above 360° (Found: C, 72·0; H, 3·5; N, 2·5. $C_{35}H_{19}O_8N$ requires C, 72·3; H, 3·3; N, 2·4%). 1 : 1′-Dianthraquinonyl-4 : 4′-dicarboxylic acid was obtained by boiling this salt with dilute hydrochloric acid. It did not melt below 360° (Found: C, 71·9; H, 3·2. Calc. for $C_{30}H_{14}O_8$: C, 71·7; H, 2·8%).

meso*Benzodianthrone*-6 : 6′-*dicarboxylic Acid*.—Finely divided copper powder (1 g.) was stirred with a solution of 1 : 1′-dianthraquinonyl-4 : 4′-dicarboxylic acid (1 g.) in concentrated sulphuric acid (30 c.c.) at 50—60°. The mixture turned green, and after 20—25 minutes it was poured over crushed ice. A yellow solid separated. The suspension was heated to boiling, then filtered, and the washed residue extracted by means of dilute sodium hydroxide solution. The filtered extract gave a brown gelatinous precipitate when acidified. The suspension was boiled, and the precipitate collected, washed, and dried.

It crystallised from nitrobenzene in small brown needles, m. p. 325° (Found: C, 76·4; H, 3·1. $C_{30}H_{14}O_6$ requires C, 76·6; H, 3·0%). meso*Benzodianthrone*-6 : 6′-*dicarboxylic acid* dissolved with a green colour in alkaline hyposulphite (dithionite). When the solution was boiled it became yellowish-brown even in the presence of an excess of alkaline hyposulphite.

Detection of 6 : 6′-*Di*meso*benzanthronyl in Mixtures with* meso*Benzanthrone and* 4 : 4′-*Di*meso*benzanthronyl.*—The acid product obtained by oxidising *meso*benzanthrone, 4 : 4′-*dimeso*benzanthronyl, or mixtures of these with chromium trioxide dissolved in concentrated sulphuric acid with a yellow colour which did not change in the presence of copper powder. The following mixture, *viz.*, *meso*benzanthrone (40%), 4 : 4′-*dimeso*benzanthronyl (50%), and 6 : 6′-*dimeso*benzanthronyl (10%) yielded a mixture of acids which gave a green solution in concentrated sulphuric acid containing copper powder; so also did the mixtures in which the proportion of 6 : 6′-*dimeso*benzanthronyl was not less than 2·5%.

*Composition of the Crude Di*meso*benzanthronyl obtained by the Action of Basic Condensing Agents on* meso*Benzanthrone.*—The crude di*meso*benzanthronyl (1 g.) obtained from *meso*benzanthrone by the action of alcoholic potassium hydroxide (Lüttringhaus and Neresheimer, *loc. cit.*) was suspended in 20 c.c. of warm glacial acetic acid and treated gradually with chromium trioxide (2·5 g.) dissolved in 5 c.c. of 50% acetic acid. When the initial rapid evolution of carbon dioxide had ceased the solution was boiled for five hours. The acid products were isolated, dissolved in concentrated sulphuric acid, and stirred with copper powder. There was no change in the initial colour of the solution, indicating that the original crude di*meso*benzanthronyl contained less than 2·5% of 6 : 6′-di*meso*benzanthronyl.

A similar result was obtained using the crude di*meso*benzanthronyl prepared by the use of sodium anilide [I.G., D.R.-P. 407,838; Frdl., **14**, 892 (1922)].

Experiments with 4-*Substituted* meso*Benzanthrones.*—(1) 4-*Methyl*meso*benzanthrone.* Potassium hydroxide was dissolved in absolute alcohol and then the solution was evaporated until 15 g. of alcohol remained. The residual paste was stirred with 4-methyl*meso*benzanthrone (3 g.) at 100—105° for six hours, sufficient alcohol being added from time to time to keep the mass pasty. The product was isolated by adding water, heating to boiling, and filtering. The washed residue was dried, and 1 g. of it was mixed with glacial acetic acid (20 c.c.) and oxidised by means of chromium trioxide (3 g.) and 50% acetic acid (6 c.c.) at the boiling point for 5 hours. The acidic products were isolated and tested by means of concentrated sulphuric acid and copper. There was no development of a green colour.

(2) 4-*Fluoro*meso*benzanthrone.* The diazonium sulphate, prepared from 4-amino*meso*benzanthrone by dissolving 5 g. of the base in 20 c.c. of concentrated sulphuric acid and stirring with a solution of sodium nitrite (2 g.) in concentrated sulphuric acid (20 c.c.), was precipitated by adding it to water, collected, washed with a small volume of water, and then dissolved in the minimum volume of water. The solution was added to one of boric acid (5 g.) in 40% hydrofluoric acid (20 g.). The diazonium fluoborate separated in the form of an orange precipitate. After being stirred for 2 hours the precipitate was collected and washed. The first washings were almost colourless, later red, and finally almost colourless. The remaining solid was washed with alcohol then with ether and lastly dried in a vacuum. The product was heated slowly to 140—150° until evolution of gas ceased. The resulting black mass was extracted by means of boiling acetic acid. The extracts deposited coloured material on cooling, and this was removed by filtration. The clear filtrate, added to water, gave a yellow precipitate. The precipitate was collected, washed, dried, and sublimed under reduced pressure. Yellow needles, m. p. 186—187°, were obtained (Found: C, 82·2; H, 3·7. $C_{17}H_9OF$ requires C, 82·2; H, 3·7%).

To a paste prepared by dissolving 10 g. of potassium hydroxide in absolute alcohol and evaporating until 5 g. of the solvent remained, was added 1 g. of the above 4-*fluoro*meso*benzanthrone.* The mixture was stirred at 105—110° for 7 hours, small successive amounts of alcohol being added to keep the paste fluid. Water was then added, and the resulting suspension boiled and filtered (for residue, see below). The deep yellow filtrate showed a green fluorescence. Acidified with hydrochloric acid, it afforded a yellow gelatinous precipitate which, when collected, washed, and dried, had m. p. 300—304° (yield, 0·7 g.). It was identified as 4-hydroxy*meso*benxanthrone by treatment with acetic anhydride and sodium acetate to give 4-acetoxy*meso*benzanthrone, m. p. 195—198°, not depressed when mixed with an authentic sample.

The alkali-insoluble portion of the product of the above reaction was oxidised by suspending 0·3 g. in 7 c.c. of glacial acetic acid and adding 5 c.c. of a solution prepared by dissolving 1 g. of chromium trioxide in 8 c.c. of 50% acetic acid. The mixture was boiled for four hours, then added to water, the suspension boiled, and the solid collected and extracted by means of dilute sodium hydroxide solution. The filtered alkaline solution gave a reddish precipitate when acidified. This was collected, redissolved in dilute alkali, and recovered by acidifying the filtered solution. When the acid product was dissolved in concentrated sulphuric acid and copper powder added there was no development of a green colour.

The experiments described in the present paper were carried out in the Department of Applied Chemistry, College of Technology, Manchester. The authors are indebted to Prof. J. Kenner, F.R.S., for providing facilities for the work, and thank the University of Bombay for the award of a Sir Mangaldas Nathubai Scholarship, and I.C.I. (Dyestuffs) Ltd. for a grant and gifts of intermediates.

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331. *Anthraquinone Series. Part II. Nitro- and Amino-2-tert.-butyl- and -n-butyl-anthraquinones, and Derived Blue Acid Dyes.*

By R. J. Moualim and A. T. Peters.

A comparison of the derivatives of 2-*tert.*- (I) and 2-*n*-butylanthraquinone (II) shows the steric influence of the *tert.*-butyl group. Compound (I) could not be halogenated, and its *mononitro*-derivative is prepared with difficulty, whereas *chloro-*, *bromo-*, and *nitro*-derivatives of (II) are prepared readily. Unlike the tert.-*butyl* analogue, 1-*amino*-2-n-*butylanthraquinone* is readily acetylated; 1 : 5-*diamino*-2-tert.-*butylanthraquinone* affords a *monoacetyl* derivative only, whilst 1 : 5-*diamino*-2-n-*butylanthraquinone* gives a *diacetyl* derivative. Blue acid dyes for wool are obtained by converting 4-*bromo*-1-*amino*-2-tert.- and -2-n-*butylanthraquinone* into the respective 4-p-*toluidino*-derivatives, and sulphonating.

2-*tert.*-Butyl- (I) and 2-*n*-butyl-anthraquinone (II) were prepared in 75% and 72% yield, respectively, by direct cyclisation of the corresponding 2-(4'-butylbenzoyl)benzoic acid with 20% fuming sulphuric acid at 95° and 100°, respectively (Peters and Rowe, *J.*, 1945, 181). No derivative of (I) is recorded in the literature, and only the mono-nitro- and -amino-derivatives of (II) have been described (Harris, Marriott, and Smith, *J.*, 1936, 1838). The preparation, properties, and orientation of derivatives of (I) and (II) are now investigated, with special reference to the differing effects of the *tert.*- and *n*-butyl groups.

Nitration of 2-*tert.*-butylanthraquinone with nitric acid (*d* 1·42 or 1·5), alone or with acetic acid or anhydride, or sulphuric acid, or with diacetylorthonitric acid (Pictet and Genequand, *Ber.*, 1902, **35**, 2526), at various temperatures, yielded resinous products, but the use of potassium nitrate (1·1 mols.) in concentrated sulphuric acid at room temperature gave 46·2% of 1-*nitro*-2-tert.-*butylanthraquinone* (III). Repeated crystallisation from acetic acid was necessary in order to obtain a pure sample, as resin formation during nitration could not be completely avoided. Reduction of (III) by boiling aqueous–alcoholic sodium sulphide yielded 1-*amino*-2-tert.-*butylanthraquinone* (IV). The position accorded to the amino-group is supported by the formation of (IV) from (III) by aqueous ammonia (*d* 0·88) at 130° in a sealed tube, and by the fact that the *tert.*-butyl group inhibits formation of a crystalline acetyl or benzoyl derivative from (IV); diazotisation is accompanied by tar formation, but an *azo*-β-*naphthol* derivative was prepared. Further evidence that the amino-group is in the 1-position is afforded by bromination to the red 4-*bromo*-1-*amino*-2-tert.-*butylanthraquinone* (V) and its conversion into the deep blue 4-p-*toluidino*-analogue (VI), which on sulphonation yielded a blue acid wool dye. The amine (IV) condensed with 1-chloroanthraquinone in presence of sodium acetate and cuprous chloride to give a good yield of 2-tert.-*butyl-di*-1 : 1'-*anthraquinonylimide* (VII).

Dinitration of 2-*tert.*-butylanthraquinone gave 66·5% of 1 : 5-*dinitro*- (VIII) and 8·7% of 1 : 8-*dinitro*-2-tert.-*butylanthraquinone*, some (VIII) also being formed during mononitration of (I). Aqueous–alcoholic sodium sulphide reduction of the respective dinitro-compound afforded 1 : 5- (IX) and 1 : 8-*diamino*-2-tert.-*butylanthraquinone*. Orientation is based partly on analogy with the dinitration of anthraquinone and 2-methylanthraquinone. The steric effect of the *tert.*-butyl group in the diamine (IX) allowed the formation of a *monoacetyl* derivative only, whereas 1 : 5-diamino-2-*n*-butylanthraquinone readily gave a diacetyl derivative (see below).

1 : 5-*Di*-α-*anthraquinonylimino*-2-tert.-*butylanthraquinone* was formed in 78% yield from the diamine (IX), and a stabilised diazo-salt prepared from (IX) showed very similar properties to the analogue derived from 1 : 5-diaminoanthraquinone, both being only slightly decomposed after 3 hours at 95°. Moreover, bromination of the diamine (IX) gave a *tribromo*-derivative, which on refluxing with *p*-toluidine in presence of sodium acetate afforded probably 6-*bromo*-1 : 5-*diamino*-4 : 8-*di*-p-*toluidino*-2-tert.-*butylanthraquinone*; subsequent disulphonation gave a bluish-green dye for wool, with similar properties to that prepared by analogous means from 1 : 5-diaminoanthraquinone.

1-Nitro-2-*n*-butylanthraquinone, prepared in 70·6% yield from 2-*n*-butylanthraquinone, was readily reduced to the corresponding amine, which afforded an *acetyl* derivative (cf. the *tert.*-butyl analogue) and an *azo*-β-*naphthol* derivative, and condensed with 1-chloroanthraquinone to give the corresponding *anthrimide*. The red 4-*bromo*-1-*amino*-2-n-*butylanthraquinone* was converted into the blue p-*toluidino*-analogue, and thence into the sulphonic acid, which dyes wool a deep blue shade, brighter than that given by the *tert.*-butyl analogue.

Dinitration of 2-*n*-butylanthraquinone yielded 61·5% of 1 : 5-dinitro- and 21% of 1 : 8-*dinitro*-2-n-*butylanthraquinone*, both being readily reduced to the corresponding 1 : 5- and 1 : 8-*diamines*, which afford *diacetyl* derivatives.

Under a variety of conditions, chlorination or bromination of 2-*tert.*-butylanthraquinone was not achieved, whereas the 2-*n*-butyl isomeride gave 1-*chloro*- and -*bromo*-2-n-*butylanthraquinone* and a *dibromo*-derivative.

In general, derivatives of 2-*tert.*- and -*n*-butylanthraquinone are more soluble in organic solvents, and have a lower m. p., than the corresponding anthraquinone derivatives.

Experimental.

1-*Nitro*-2-tert.-*butylanthraquinone* (III).—Powdered potassium nitrate (12·2 g.; 1·2 mols.) was added during 15 minutes to a vigorously stirred solution of 2-*tert.*-butylanthraquinone (26·4 g.; 1 mol. in concentrated sulphuric acid (200 c.c.) at room temperature. After 6 hours' stirring, the mixture was left overnight and a small amount of insoluble dinitro-compound collected. Addition of the filtrate to ice–water gave a pale yellow solid, m. p. 115—120° (decomp., forming an opaque mass, which clarified at 160°); boiling with aqueous sodium hydrogen sulphite did not effect further purification, which was best carried out by repeated crystallisation from acetic acid, giving pale yellow needles, of constant m. p. 206—207°, of the *mononitro*-compound (III) (14·3 g.; 46·2%) (Found: C, 69·3; H, 4·9; N, 4·6. $C_{18}H_{15}O_4N$ requires C, 69·9; H, 4·85; N, 4·5%). No further pure nitro-derivative was isolable from the acetic acid mother liquors.

The insoluble *dinitro*-derivative crystallised from much acetic acid in almost colourless needles, m. p. 315° (2·2 g.; 6·2%) (Found: C, 60·5; H, 4·0; N, 7·6. $C_{18}H_{14}O_6N_2$ requires C, 61·0; H, 4·0; N, 7·9%) (see below).

1-*Amino*-2-tert.-*butylanthraquinone* (IV).—(*a*) Pure 1-nitro-2-*tert.*-butylanthraquinone (3 g.) was refluxed with sodium sulphide crystals (15 g.) in 50% aqueous alcohol (70 c.c.) for 40 minutes. On careful addition of water, the *amine* separated as a red solid; it crystallised from 90% alcohol in scarlet prismatic needles, m. p. 161° (2·3 g.; 85·2%) (Found: C, 77·5; H, 6·0; N, 5·1. $C_{18}H_{17}O_2N$ requires C, 77·4; H, 6·1; H, 5·0%). The amine gives a colourless sulphate with concentrated sulphuric acid, but it is a weak base, dilution quickly hydrolysing the salt. It is diazotisable, but attempted acetylation or benzoylation in presence of pyridine or nitrobenzene at various temperatures afforded tarry products which could not be crystallised. The crude nitration product (5·9 g.), m. p. 115—120° (decomp.), was reduced as above to yield dark brownish-red nodules, m. p. 70° (4·5 g.; 84·5%), which were chromatographed using alumina and benzene; the only pure product isolated was the above amine, m. p. and mixed m. p. 160—161°.

(*b*) 1-Nitro-2-*tert.*-butylanthraquinone (1 g.), m. p. 206—207°, was heated with aqueous ammonia (*d* 0·88; 20 c.c.) at 130° for 6 hours in a sealed tube. The brownish-red mass crystallised from 90% alcohol in red prismatic needless, m. p. 161° (0·4 g.), not depressed on admixture with the amine obtained by method (*a*).

Derivatives of the Amine (IV).—Diazotisation in cold concentrated sulphuric acid was not neat, there being some tar formation, but subsequent coupling with alkaline β-naphthol gave 35% of the *azo-β-naphthol* derivative, which crystallised from acetic acid in reddish-brown prismatic needles, m. p. 222° (Found: N, 6·1. $C_{28}H_{22}O_3N_2$ requires N, 6·5%), soluble in cold concentrated sulphuric acid to give a blue solution.

The amine (1 g.; 1 mol.) was refluxed with 1-chloroanthraquinone (0·95 g.; 1·1 mols.), anhydrous sodium acetate (1·2 g.), cuprous chloride (0·2 g.), and nitrobenzene (16 c.c.) for 6 hours. Dilution with alcohol (40 c.c.) gave a precipitate of 2-tert.-*butyl-di*-1 : 1′-*anthraquinonylimide* (1·5 g.; 86·2%), which crystallised from benzene in brownish-red prismatic needles, m. p. >360° (Found: N, 2·6. $C_{32}H_{23}O_4N$ requires N, 2·9%).

4-*Bromo*-1-*amino*-2-tert.-*butylanthraquinone* (V).—1-Amino-2-*tert.*-butylanthraquinone (2 g.) was dissolved in nitrobenzene (15 c.c.), and fused sodium acetate (0·7 g.) added. Bromine (1·8 g.; 1·5 mols.) in nitrobenzene (6 c.c.) was added at 8°, and, after 30 minutes' stirring at room temperature, the temperature was raised gradually to 95° and kept there for 1 hour. A little solid (0·2 g.) separated, and was collected, washed with aqueous sodium carbonate and then alcohol, and crystallised from nitrobenzene to give glistening, scarlet prismatic needles, decomp. at *ca.* 320° (Found: Br, 47·0. $C_{18}H_{14}O_2NBr_3$ requires Br, 46·5%), of a *tribromo*-derivative.

After removal of the nitrobenzene with steam, the main reaction product of 4-*bromo*-1-*amino*-2-tert.-*butylanthraquinone* crystallised from alcohol in red prismatic needles, m. p. 158° (2 g.; 78·2%) (Found: C, 60·0; H, 4·2; N, 4·2; Br, 22·6. $C_{18}H_{16}O_2NBr$ requires C, 60·3; H, 4·5; N, 3·9; Br, 22·3%).

1-*Amino*-4-p-*toluidino*-2-tert.-*butylanthraquinone* (VI).—The above 4-bromo-compound (1 g.) was heated with *p*-toluidine (3 g.), anhydrous sodium acetate (1 g.), and copper acetate (0·1 g.) at 95° for 16 hours. Alcohol (20 c.c.) was added; the separated 4-p-*toluidino*-derivative (VI) crystallised from pyridine in deep blue needles, m. p. 135° (0·7 g.; 65·1%) (Found: C, 77·6; H, 5·6; N, 7·9. $C_{25}H_{24}O_2N_2$ requires C, 78·1; H, 6·2; N, 7·3%). Confirmation that the compound had the constitution assigned was afforded by sulphonation with 5% fuming sulphuric acid at 30° for 6 hours; on salting out the product from aqueous solution with sodium sulphate, a sulphonic acid was obtained which dyes wool from an acetic acid and sodium sulphate bath a blue shade of similar light fastness to that shown by the 2-methyl analogue, *viz.*, Solway Blue RNS.

Dinitration of 2-tert.-*Butylanthraquinone*: 1 : 5- *and* 1 : 8-*Dinitro-compounds*.—Powdered potassium nitrate (10 g.; 2·2 mols.) was added during 15 minutes to a solution of 2-*tert.*-butylanthraquinone (12 g.) in concentrated sulphuric acid (140 c.c.) at 40°; after 6 hours' stirring at this temperature, the precipitate was collected; the 1 : 5-*dinitro*-2-tert.-*butylanthraquinone* crystallised from acetic acid in almost colourless needles, m. p. 315° (10·7 g.; 66·5%), identical with the dinitro-compound formed during mononitration (above). The 1 : 5-dinitro-derivative is very sparingly soluble in alcohol.

The sulphuric acid filtrate was poured on ice, and the resulting solid crystallised several times from alcohol to yield pale yellow needles, m. p. 170° (1·6 g.; 8·7%), of the 1 : 8-*dinitro*-isomeride (Found: C, 60·1; H, 3·9; N, 7·7. $C_{18}H_{14}O_6N_2$ requires C, 61·0; H, 4·0; N, 7·9%).

1 : 5- *and* 1 : 8-*Diamino*-2-tert.-*butylanthraquinone*.—The respective dinitro-compounds (2 g.) were

reduced by refluxing them with sodium sulphide crystals (20 g.) in 50% aqueous alcohol (100 c.c.) for 1 hour.

1 : 5-*Diamino*-2-tert.-*butylanthraquinone* crystallised from alcohol in glistening red leaflets, m. p. 158° (1·7 g.; 91%) (Found: C, 72·9; H, 5·95; N, 9·5. $C_{18}H_{18}O_2N_2$ requires C, 73·4; H, 6·1; N, 9·5%). It is strongly basic, and dissolves in hydrochloric acid to give a pale yellow solution, and is easily diazotisable to yield stable diazo-solutions.

The 1 : 5-diamine (0·5 g.) was dissolved in concentrated hydrochloric acid (3 c.c.) and sodium nitrite (0·75 g.) added slowly at 10°, with stirring. After 30 minutes, anhydrous sodium sulphate (2·5 g.) was added and the pale brown diazonium salt was collected and dried (2·3 g., 26·5% strength; 92%). After 3 hours' heating at 35°, 55°, 75°, and 95°, the strengths as determined by titration with alkaline β-naphthol were 26·5, 26, 25, and 24·4%, respectively, indicating very good stability to heat; in this respect, the diamine resembles 1 : 5-diaminoanthraquinone.

A stabilised diazo-compound was also readily formed as the zinc chloride double salt.

The *bisazo-β-naphthol* derivative crystallised from benzene in bordeaux needles, with a green reflex, m. p. 298° (Found: N, 9·5. $C_{38}H_{26}O_4N_4$ requires N, 9·3%), which dissolve in cold concentrated sulphuric acid with a violet colour.

On refluxing with acetic anhydride for 4 hours, the 1 : 5-diamine gave the *monoacetyl* derivative, which separated in brick-red glistening prismatic needles; crystallisation from alcohol gave deep claret prisms, m. p. 244° (Found: C, 70·1; H, 6·4; N, 8·2; Ac, 13·7. $C_{22}H_{22}O_4N_2$ requires C, 69·8; H, 5·8; N, 7·4; Ac, 22·8. $C_{20}H_{20}O_3N_2$ requires C, 71·4; H, 6·0; N, 8·3; Ac, 12·9%).

1 : 5-Diamino-2-*tert.*-butylanthraquinone (0·4 g.) was refluxed with 1-chloroanthraquinone (0·8 g.), anhydrous sodium acetate (0·3 g.), cuprous chloride (0·1 g.), and nitrobenzene (6 c.c.) for 6 hours; the precipitate which separated on cooling crystallised from benzene in bordeaux plates, m. p. >360° (0·7 g.; 78%), of 1 : 5-*di-α-anthraquinonylimino*-2-tert.-*butylanthraquinone* (Found: N, 4·2. $C_{46}H_{30}O_6N_2$ requires N, 3·9%), soluble in cold concentrated sulphuric acid to give a deep blue solution.

1 : 8-*Diamino*-2-tert.-*butylanthraquinone* crystallised from alcohol in reddish-brown needles, m. p. 143° (1·4 g.; 75·3%) (Found: C, 72·8; H, 5·8; N, 9·3. $C_{18}H_{18}O_2N_2$ requires C, 73·4; H, 6·1; N, 9·5%), difficultly soluble in hydrochloric acid and not readily diazotisable.

(?) 4 : 6 : 8-*Tribromo*-1 : 5-*diamino*-2-tert.-*butylanthraquinone, and its Conversion into a Bluish-green Wool Dye.*—The 1 : 5-diamine (1 g.) was treated in acetic acid solution (100 c.c.) with excess of bromine (5 g.) and the mixture was refluxed for 3 hours; the deep red product which separated was washed with warm aqueous sodium carbonate; it crystallised from nitrobenzene in claret needles, m. p. >360° (1·3 g.; 71·4%) (Found: Br, 45·6. $C_{18}H_{15}O_2N_2Br_3$ requires Br, 45·2%), of the *tribromo*-compound. On refluxing 1 g. with *p*-toluidine (10 g.) and sodium acetate (2 g.) for 6 hours, the colour changed through violet to bluish-green; after cooling, the resulting precipitate was collected, washed well with dilute hydrochloric acid, and then aqueous sodium carbonate and water; it crystallised from pyridine in deep bluish-green needles, m. p. 260—261° (0·7 g.; 63·6%) (Found: N, 9·5; Br, 14·2. $C_{32}H_{21}O_2N_4Br$ requires N, 9·7; Br, 13·7%).

(?) 6-*Bromo*-1 : 5-*diamino*-4 : 8-*di*-p-*toluidino*-2-tert.-*butylanthraquinone* was sulphonated with 100% sulphuric acid at 30° for 20 minutes, and the disulphonic acid purified by careful salting out from aqueous solution with sodium sulphate. It dyes wool a bright greenish-blue shade, similar to that given by the analogous 2-methyl derivative.

Attempted Halogenation of 2-tert.-*Butylanthraquinone.*—Direct chlorination in various solvents, or the use of sulphuryl chloride in nitrobenzene (with a little iodine) at 95° for 6 hours, gave unchanged material; sulphuryl chloride and nitrobenzene in a sealed tube at 160° for 5 hours afforded a resin.

2-*tert.*-Butylanthraquinone was unchanged with bromine in nitrobenzene in presence of sodium acetate at 95—160° for 4 hours, and bromination in 20% fuming sulphuric acid at 100—130° gave a resin.

1-*Nitro*-2-n-*butylanthraquinone.*—Potassium nitrate (6·3 g.; 1·2 mols.) was added to 2-*n*-butylanthraquinone (13·2 g.; 1 mol.) in concentrated sulphuric acid (100 c.c.) during 20 minutes, at room temperature, and the mixture was stirred at 45° for 6 hours and left overnight at room temperature. The solid which had separated crystallised from acetic acid in pale yellow needles, m. p. 260°, of 1 : 5-dinitro-2-*n*-butylanthraquinone (2·5 g.; 14·1%) (see below). Dilution of the sulphuric acid filtrate gave 1-nitro-2-*n*-butylanthraquinone, which crystallised from acetic acid in pale yellow needles, m. p. 150° (10·9 g.; 70·6%) (Found: C, 70·0; H, 4·7; N, 4·3. Calc. for $C_{18}H_{15}O_4N$: C, 69·9; H, 4·85; N, 4·5%).

1-*Amino*-2-n-*butylanthraquinone.*—The above mononitro-compound (5 g.) was refluxed with sodium sulphide crystals (30 g.) in 50% aqueous alcohol (120 c.c.) for 1 hour, and, on addition of water, the amine separated; it crystallised from aqueous alcohol in long, red prismatic needles, m. p. 172° (4·2 g.; 91·3%) (Found: C, 77·1; H, 6·0; N, 5·0. Calc. for $C_{18}H_{17}O_2N$: C, 77·4; H, 6·1; N, 5·0%). Its *acetyl* derivative separated from acetic anhydride (boil for 3 hours), and crystallised from 50% aqueous alcohol in yellow needles, m. p. 140° (Found: N, 4·3. $C_{20}H_{19}O_3N$ requires N, 4·4%). The amine was diazotised in sulphuric acid, and afforded an *azo-β-naphthol* derivative, which crystallised from acetic acid in brownish-red plates, m. p. 243° (Found: N, 6·0. $C_{28}H_{22}O_3N_2$ requires N, 6·5%).

2-n-*Butyl-di*-1 : 1′-*anthraquinonylimide*, prepared by heating with 1-chloroanthraquinone, as above, crystallised from benzene in brownish-red needles, m. p. >360° (Found: N, 2·7. $C_{32}H_{23}O_4N$ requires N, 2·9%).

4-*Bromo*-1-*amino*-2-n-*butylanthraquinone.*—Prepared in similar manner to that used for the *tert.*-butyl analogue, the 4-*bromo*-derivative formed red prismatic needles (alcohol), m. p. 148° (78%) (Found: N, 4·5; Br, 22·3. $C_{18}H_{16}O_2NBr$ requires N, 4·5; Br, 22·3).

1-*Amino*-4-p-*toluidino*-2-n-*butylanthraquinone.*—This *compound* crystallised from pyridine in blue needles, m. p. 145° (72%) (Found: C, 78·4; H, 5·5; N, 7·7. $C_{25}H_{24}O_2N_2$ requires C, 78·1; H, 6·2; N, 7·3%), which on sulphonation at 30°, as above, yielded a sulphonic acid which dyes wool from an acetic acid and Glauber's salt bath a deep blue shade brighter than that afforded by the *tert.*-butyl analogue.

Dinitration of 2-n-*Butylanthraquinone*: 1 : 5- *and* 1 : 8-*Dinitro-compounds.*—Potassium nitrate (7 g.; 2·2 mols.) was added during 20 minutes to a stirred solution of 2-*n*-butylanthraquinone (8 g.;

1 mol.) in concentrated sulphuric acid (100 c.c.) at 40°. After 6 hours' stirring at this temperature, the crystals were collected; recrystallisation from acetic acid gave yellow needles, m. p. 260° (6·4 g.; 61·5%) (Found: C, 61·4; H, 4·0; N, 7·7. $C_{18}H_{14}O_6N_2$ requires C, 61·0; H, 4·0; N, 7·9%), of 1 : 5-*dinitro*-2-n-*butylanthraquinone*.

The sulphuric acid filtrate was poured on ice, and the yellow precipitate crystallised several times from alcohol, to yield yellow needles, m. p. 158°, of 1 : 8-*dinitro*-2-n-*butylanthraquinone* (2·2 g.; 21·1%) (Found: C, 61·5; H, 3·9; N, 7·8%).

1 : 5- *and* 1 : 8-*Diamino*-2-n-*butylanthraquinone*.—The 1 : 5-diamine crystallised from alcohol in large orange-red needles, m. p. 160° (90%) (Found: C, 72·9; H, 6·3; N, 9·2. $C_{18}H_{18}O_2N_2$ requires C, 73·4; H, 6·1; N, 9·5%).

The 1 : 5-diamine readily formed a stable diazonium salt (cf. the *tert.*-butyl analogue), which was very stable to heat; thus, a salt of 19% strength on being heated at 75° or 95° for 3 hours was reduced in strength to 18 or 17·6%, respectively. A stable *iso*diazoate was also formed readily.

The *bisazo-β-naphthol* derivative crystallised from benzene in brownish-red needles, m. p. 298—300° (Found: N, 8·7. $C_{38}H_{28}O_4N_4$ requires N, 9·2%).

With boiling acetic anhydride for 3 hours, the 1 : 5-diamine gave a *diacetyl* derivative, which crystallised from alcohol in yellow needles, m. p. 182° (Found: N, 8·0; Ac, 24·4. $C_{22}H_{22}O_4N_2$ requires N, 7·4; Ac, 22·8%).

The *dianthrimide*, prepared by refluxing the 1 : 5-diamine with 1-chloroanthraquinone as above, crystallised from benzene in deep claret needles, m. p. >360° (Found: N, 4·3. $C_{46}H_{30}O_6N_2$ requires N, 3·9%), which dissolved in cold concentrated sulphuric acid to give a deep blue solution.

1 : 8-*Diamino*-2-n-*butylanthraquinone* crystallised from alcohol in reddish-brown needles, m. p. 140° (Found: N, 9·3. $C_{18}H_{18}O_2N_2$ requires N, 9·5%), and gave a *diacetyl* derivative, which crystallised from alcohol in yellowish-brown needles, m. p. 152° (Found: N, 7·9. $C_{22}H_{22}O_4N_2$ requires N, 7·4%).

1-*Chloro*-2-n-*butylanthraquinone*.—2-*n*-Butylanthraquinone (1·5 g.) was heated gradually with sulphuryl chloride (1 g.), iodine (0·2 g.), and nitrobenzene (8 c.c.), and finally heated under reflux on the water-bath for 5 hours. The nitrobenzene was removed with steam; the residue of *chloro*-compound crystallised from alcohol in cream needles, m. p. 74° (1·2 g.; 69%) (Found: Cl, 11·4. $C_{18}H_{15}O_2Cl$ requires Cl, 11·9%).

1-*Bromo*-2-n-*butylanthraquinone*.—Bromine (2·5 g.; 1·2 mols.) was added to a solution of 2-*n*-butylanthraquinone (3 g.; 1 mol.) in nitrobenzene (15 c.c.), in presence of anhydrous sodium acetate (1 g.) at 8°, and the temperature was raised gradually to 95° and kept there for 3 hours. The mixture was added to alcohol (40 c.c.) and left overnight; the precipitate crystallised from nitrobenzene in yellow needles, m. p. 149—150°, of a *dibromo*-derivative (0·5 g.; 10·4%) (Found: C, 51·6; H, 3·6; Br, 37·3. $C_{18}H_{14}O_2Br_2$ requires C, 51·2; H, 3·3; Br, 37·9%). After removal of the solvent from the alcohol-nitrobenzene filtrate by steam distillation, the resulting yellow solid crystallised from alcohol in yellow needles, m. p. 95°, of 1-*bromo*-2-n-*butylanthraquinone* (1·7 g.; 43·8%) (Found: C, 62·2; H, 4·2; Br, 24·2. $C_{18}H_{15}O_2Br$ requires C, 63·0; H, 4·4; Br, 23·3%). Analysis suggested that a little of the dibromo-compound was present.

Micro-analyses were carried out by Drs. G. Weiler and F. B. Strauss, of Oxford. The authors thank Imperial Chemical Industries Ltd., Dyestuffs Division, for gifts of chemicals, and Dr. S. Coffey for his kind co-operation and interest in this work.

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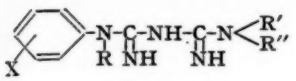
332. *Synthetic Antimalarials. Part XXVIII. An Alternative Route to* N^1-*Aryl*-N^5-*alkyldiguanides.*

By F. H. S. CURD, J. A. HENDRY, T. S. KENNY, A. G. MURRAY, and F. L. ROSE.

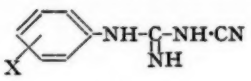
Part X (*J.*, 1946, 729) of this series described the preparation of N^1-aryl-N^5-alkyl- and -dialkyl-diguanides, for examination as antimalarial agents, by the interaction of alkyl- or dialkyl-amines with aryldicyandiamides. The latter were prepared from the triazenes resulting from the coupling of diazonium salts with dicyandiamide. Their preparation is now described from arylamines and dicyanimide. Alkylamines or dialkylamines with dicyanimide analogously give alkyl- or dialkyl-dicyandiamides which react with arylamines to yield the N^1 : N^5-substituted diguanides of the same type. Alkyldicyandiamides have also been prepared by amination of the *S*-alkyl*iso*thioureas resulting from alkylation of the *N*-cyanothioureas obtained by reaction of alkyl *iso*thiocyanates with cyanamide.

IN Part X of this series (*J.*, 1946, 729) there was reported the preparation of a number of N^1-aryl-N^5-alkyldiguanides of type (I; R = H or Me) possessing antimalarial activity, and the selection of one member of the series (I; X = *p*-Cl, R = R′ = H, R″ = Pr$^\beta$) ("Paludrine") for use in the clinical treatment of human malaria was noted. These substances were all made by the reaction of an aryldicyandiamide (II) with an amine either as a salt of a mineral acid or in the presence of an agent such as copper sulphate, in which case the copper complex of the diguanide was formed. The aryldicyandiamides were themselves prepared through the intermediate triazenes (III) obtained by coupling the appropriate diazonium salt with

dicyandiamide. To obtain the diguanides (I; R = Me) the intermediate aryldicyandiamide was treated in alkaline solution with methyl sulphate. This reaction series often gave



(I.)



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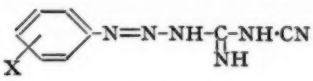
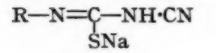


(III.)

satisfactory yields of the products required, but the route had too many limitations for convenient use in the extensive exploration of the relationship between antimalarial activity and the many chemical modifications of which the diguanides should be capable. For example, not all primary aromatic amines could be diazotised and coupled with dicyandiamide, and the resultant triazenes converted into aryldicyandiamides in good yield, and furthermore the method either did not allow or appeared unsuitable for the preparation of derivatives carrying substituents, such as further aryl and alkyl groups, on N^2, N^3, and N^4. For this reason many alternative preparative procedures have been examined which it is proposed to report in later papers of this series. In this, the second communication on diguanides, only those routes based on N^1-substituted dicyandiamide derivatives (VI) are discussed.

The first alternative route investigated was the converse of that described in Part X (*loc. cit.*), namely, the preparation of alkyldicyandiamides and their reaction with arylamines. This method appeared to be particularly suitable for the synthesis of N^1-aryl-N^5-alkyldiguanides variously substituted in the aromatic ring, since it was independent of the capacity of the aniline to diazotise and couple with dicyandiamide. No monoalkyldicyandiamides had been recorded in the literature, but it was thought that they should be accessible by a route analogous to that first employed by Wheeler and Jamieson (*J. Amer. Chem. Soc.*, 1903, **25**, 719) for the preparation of phenyldicyandiamide. In this synthesis, phenyl *iso*thiocyanate was condensed with sodium cyanamide to give (IV; R = Ph) which on successive methylation and reaction with ammonia



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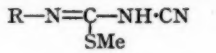

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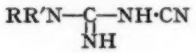


(VI.)

gave (V; R = Ph) and then (II; X = H). The condensation of sodium cyanamide with mustard oils to give (IV; R = alkyl) was first described by Wunderlich (*Ber.*, 1886, **19**, 448), and the same worker brought the products into reaction with alkyl halides to give the corresponding compounds of type (V; R = alkyl), although, as pointed out by Wheeler and Jamieson (*loc. cit.*), an incorrect structure was initially assigned to these dialkyl derivatives. Later, Hecht (*Ber.*, 1890, **23**, 1658) prepared a large number of homologues. By treatment of these compounds with ammonia, for example in alcohol at 120°, we have shown that an alkylthiol is eliminated, and in this way have prepared the *methyl-*, *ethyl-*, n- and iso-*propyl-*, and n- and iso-*butyl-dicyandiamides* (VI; R = H, R′ = alkyl). While this paper was being written May (*J. Org. Chem.*, 1947, **12**, 437) described the preparation, by a similar reaction, of (VI; R = H, R′ = Me) and (VI; R = H, R′ = Pr$^\beta$), although the latter was not isolated or characterised.

A more satisfactory route to the same alkyldicyandiamides was that using dicyanimide. Slotta and Tschesche (*Ber.*, 1929, **62**, 1394) caused this substance to react with two molecular proportions of alkylamines at 120—130°, and obtained the symmetrical N^1 : N^5-dialkyldiguanides. They do not record the intermediate production of alkyldicyandiamides. In a similar way we have prepared N^1 : N^5-di*iso*propyldiguanide, but we have also found that by the use of one molecular proportion of an alkylamine alkyldicyandiamides can be prepared in good yield. This route is not only simple, but makes it possible to obtain dialkyl dervatives of type (VI; R = R′ = Me, Et; R = Me, R′ = Pr$^\beta$; RR′ = $[CH_2]_5$) which are not available through the alkyl*iso*thioureas (V). The reaction can be effected in several ways. Thus sodium dicyanimide and *iso*propylamine hydrochloride have been condensed by heating them either in a solvent such as butanol or in an aqueous solution of such concentration that a temperature of 116—120° was achieved. Alternatively, the *iso*propylamine salt of dicyanimide was prepared by double decomposition in water of *iso*propylamine and zinc dicyanimide (U.S.P. 2,418,476), and removal of the hydrated zinc oxide. Evaporation to dryness and fusion of the resultant hygroscopic salt at 85° gave a high yield of *iso*propyldicyandiamide.

Dicyanimide has been made in a number of ways. Madelung and Kern (*Annalen*, 1922, **427**, 1) described its preparation from cyanogen bromide and disodium cyanamide, and Biechler (*Chem. Zentr.*, 1935, II, 1163) from cyanogen bromide and sodamide. The former reaction was

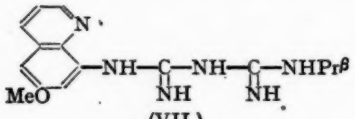
effected in aqueous solution, and the dry sodium salt obtained by evaporation. This has been used although for some purposes the aqueous reaction mixture was suitable. A convenient alternative was by adding cyanogen bromide to or passing gaseous cyanogen chloride into a solution of commercial calcium cyanamide, making the mixture slightly acid, heating it to destroy cyanate produced from a side reaction, and then using the total liquor in subsequent condensations.

Many diguanides were made by boiling alkyldicyandiamides with arylamine salts, usually the hydrochlorides, for a few hours, and isolated as the hydrochlorides which crystallised from the mixture on cooling. This method has been applied to *p*-chloroaniline, which with *iso*propyldicyandiamide gave N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (" Paludrine ") identical with the product prepared as described in Part X. β-Ethoxyethanol with or without the addition of water was a convenient alternative solvent, particularly when the arylamine salts were sparingly water-soluble.

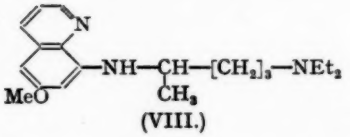
The dicyanimide route has been applied in a similar manner to the preparation of the aryldicyandiamides (II). This substance reacted more easily with arylamines than with alkylamines. Thus with *p*-chloroaniline hydrochloride in water, the reaction was approximately 80% complete after 24 hours at room temperature, or after 7 hours at 50—60°. For the preparation of the *α-naphthyl-* and NN-*diphenyl-dicyandiamides* (VI; R = H, R′ = α-$C_{10}H_7$; R = R′ = Ph) the reaction was carried out in aqueous ethanol. Several of the aryldicyandiamides described in Part X have been made by the new method, and the identity of the specimens established. In addition, the aryldicyandiamides have been condensed with alkylamines, the latter being used as salts such as the hydrochlorides, by a method which is less tedious than those described in Part X. Previous experience was that refluxing the components together in aqueous solution gave, in general, poor yields of the diguanides. Good yields are obtained, however, when water is replaced by an organic solvent which permits the reaction to be effected at a higher temperature. Typical reaction conditions are described in the experimental section for the preparation of N¹-p-*fluorophenyl*-N⁵-iso*propyldiguanide* (I; X = *p*-F, R = R′ = H, R″ = Pr$^{\beta}$) from *p*-fluorophenyldicyandiamide and *iso*propylamine hydrochloride in nitrobenzene at 130—135°.

Development of the above synthetic methods has enabled us to prepare a large number of N^1-substituted phenyl-N^5-alkyl-(and dialkyl-) diguanides and N¹-(6-*bromo*-2-*naphthyl*)-N⁵-iso*propyldiguanide* carrying the bromonaphthyl residue which conferred high antimalarial activity in the anilinopyrimidines (see Part V, *J.*, 1946, 366). We have also been enabled to prepare diguanides such as N¹-(6-*quinolyl*)-N⁵-iso*propyldiguanide hydrochloride* and N¹-(8-*chloro*-6-*quinolyl*)-N⁵-iso*propyldiguanide hydrochloride* which contain a quinoline residue in place of the substituted phenyl group of type (I), a variation that was suggested by the work described in Part XXII (*J.*, 1947, 1613) where an analogous alteration of the earlier pyrimidine type is reported.

Spinks (*Ann. Trop. Med. Parasit.*, 1947, **41**, 30) has shown that Paludrine exhibits absorption and distribution characteristics qualitatively similar to those of mepacrine and quinine. On the assumption that the basic side chain of the older antimarial compounds is primarily responsible for these properties (see Magidson *et al.*, *Arch. Pharm.*, 1934, **274**, 74; Magidson and Grigorowsky, *Ber.*, 1936, **69**, 396), this suggested that the *iso*propyldiguanide residue of Paludrine, besides being concerned with the intrinsic antimalarial activity, also simulated the dialkylaminoalkylamino-group in its effect on absorption, etc. Accordingly we have prepared N^1-(6-methoxy-8-quinolyl)-N^5-*iso*propyldiguanide (VII) (*hydrochloride*) in which the place of the basic side chain in pamaquin (VIII) is taken by an *iso*propyldiguanide residue.



(VII.)



(VIII.)

In previous papers of this series it has been customary to include brief notes recording the results obtained by our colleague, Dr. D. G. Davey, with the compounds described, in tests against avian malaria. The diguanide type of antimalarial has been the subject of very extensive biological as well as chemical researches. It is more convenient to record the preparative work here and to reserve the biological results for discussion elsewhere when they can be dealt with in a more logical manner.

Experimental.

Alkyldicyandiamides. (a) *From Dicyanimide.*

Methyldicyandiamide (VI; R = H, R′ = Me).—Methylamine hydrochloride (6·8 g.), sodium dicyanimide (8·9 g.), and butanol (50 c.c.) were refluxed with stirring for 3 hours. The cooled suspension was filtered and the filtrate evaporated. The residual syrup solidified on treatment with dioxan and the compound recrystallised from the same solvent in colourless prisms (5·5 g.), m. p. 83° (Found: C, 33·2; H, 6·5; N, 52·6. Calc. for $C_3H_6N_4,\frac{1}{2}H_2O$: C, 33·65; H, 6·55; N, 52·35%). After being dried for 48 hours over sodium hydroxide and paraffin wax in a vacuum it had m. p. 93—94° (Found: C, 36·2; H, 6·15. Calc. for $C_3H_6N_4$: C, 36·8; H, 6·1%). May (*loc. cit.*) gives m. p. 91—92°.

Ethyldicyandiamide (VI; R = H, R′ = Et).—Similarly prepared from ethylamine hydrochloride (8·2 g.), the *compound* (5·4 g.), m. p. 108—109°, retained dioxan after recrystallisation from water (Found: C, 45·45; H, 7·3; N, 36·9. $C_4H_8N_4,\frac{1}{2}C_4H_8O_2$ requires C, 46·2; H, 7·7; N, 36·0%). Prolonged drying as for the methyl homologue followed by recrystallisation from ethyl acetate–petroleum gave colourless prisms, m. p. 71—72° (Found: C, 42·85; H, 7·3; N, 49·8. $C_4H_8N_4$ requires C, 42·8; H, 7·1; N, 50·0%).

n-*Propyldicyandiamide* (VI; R = H, R′ = Pr$^{\alpha}$).—Similarly prepared from *n*-propylamine hydrochloride, itself obtained solid in the reaction flask by neutralising 4N-hydrochloric acid (25 c.c.) with *n*-propylamine and evaporating to dryness, the *compound* successively recrystallised from dioxan and water formed colourless prisms, m. p. 92—93° (Found: C, 47·75; H, 7·7; N, 44·1. $C_5H_{10}N_4$ requires C, 47·6; H, 7·95; N, 44·45%).

iso*Propyldicyandiamide* (VI; R = H, R′ = Pr$^{\beta}$).—Similarly prepared using *iso*propylamine hydrochloride (7·2 g.), sodium dicyanimide (6·7 g.), and butanol (18·5 c.c.), the *compound*, recrystallised from dioxan and dried at 40—45°, formed colourless rods, m. p. 111—113° (Found: C, 49·6; H, 8·1; N, 31·65. $C_5H_{10}N_4,\frac{1}{2}C_4H_8O_2$ requires C, 49·5; H, 8·25; N, 32·95%). Dried in a vacuum at 70—75° it had m. p. 101° (Found: C, 47·35; H, 7·45; N, 44·35. $C_5H_{10}N_4$ requires C, 47·6; H, 7·95; N, 44·45%).

iso*Butyldicyandiamide* (VI; R = H, R′ = Bu$^{\beta}$).—Similarly prepared using *iso*butylamine hydrochloride (37·5 g.), sodium dicyanimide (30 g.), and butanol (120 c.c.), the *compound*, recrystallised from water, formed flat colourless prisms, m. p. 112° (Found: C, 51·8; H, 8·5; N, 39·6. $C_6H_{12}N_4$ requires C, 51·4; H, 8·6; N, 40·0%).

cyclo*Hexyldicyandiamide* (VI; R = H, R′ = C_6H_{11}).—Similarly prepared using *cyclo*hexylamine hydrochloride (27 g.), sodium dicyanimide (18 g.), and butanol (50 c.c.), the *compound*, recrystallised from water, formed flat colourless prisms, m. p. 166° (Found: C, 58·1; H, 8·2; N, 33·6. $C_8H_{14}N_4$ requires C, 57·8; H, 8·4; N, 33·7%).

n-*Amyldicyandiamide* (VI; R = H, R′ = Am$^{\alpha}$).—Similarly prepared using *n*-amylamine hydrochloride (23·9 g.), sodium dicyanimide (18 g.), and butanol (50 c.c.), the *compound*, recrystallised first from a little chlorobenzene and then from water, formed long colourless prisms, m. p. 113—115° (Found: C, 54·5; H, 8·8; N, 36·1. $C_7H_{14}N_4$ requires C, 54·6; H, 9·1; N, 36·4%).

n-*Butyldicyandiamide* (VI; R = H, R′ = Bu$^{\alpha}$).—*n*-Butylamine hydrochloride (9·9 g.), sodium dicyanimide (9 g.), and water (2·5 c.c.) were heated at 95—100° for 4 hours, diluted with cold water (75 c.c.), and extracted with chloroform. The chloroform solution was dried (Na_2SO_4) and evaporated. The solid obtained after expressing the oil from the residue gave the *compound* as colourless prisms after crystallisation from water, m. p. 76—78° (Found: C, 51·4; H, 8·5; N, 40·0. $C_6H_{12}N_4$ requires C, 51·4; H, 8·6; N, 40·0%).

Dimethyldicyandiamide (VI; R = R′ = Me).—Prepared as described for the monomethyl homologue from dimethylamine hydrochloride (46 g.), sodium dicyanimide (37 g.), and butanol (120 c.c.), the *compound* crystallised on cooling (18 g., and a further 14 g. from evaporation of the mother liquor). It gave colourless crystals from water, m. p. 176—177° (Found: C, 42·9; H, 7·0; N, 50·0. $C_4H_8N_4$ requires C, 42·8; H, 7·1; N, 50·1%).

Diethyldicyandiamide (VI; R = R′ = Et).—Similarly prepared, the *compound* gave colourless needles from toluene, m. p. 150—151° (Found: C, 51·35; H, 8·55; N, 40·1. $C_6H_{12}N_4$ requires C, 51·4; H, 8·6; N, 40·0%).

*Methyl*iso*propyldicyandiamide* (VI; R = Me, R′ = Pr$^{\beta}$).—Prepared as described for the *n*-propyl homologue, the *compound* was obtained solid after distilling off the butanol, digesting the residue with hot water, treating it with charcoal, and cooling. It formed colourless leaflets, m. p. 64—65° (Found: C, 48·25; H, 8·85; N, 37·35. $C_6H_{12}N_4,\frac{1}{2}H_2O$ requires C, 48·3; H, 8·75; N, 37·6%).

cyclo*Pentamethylenedicyandiamide* (VI; RR′ = $[CH_2]_5$).—Piperidine (30 c.c.), 2N-hydrochloric acid (150 c.c.), and sodium dicyanimide (30 g.) were refluxed for 8 hours. The dark precipitate of the *compound* obtained on cooling when recrystallised from water with charcoal treatment formed colourless prisms, m. p. 153—155° (Found: C, 55·6; H, 7·35; N, 36·45. $C_7H_{12}N_4$ requires C, 55·3; H, 7·9; N, 36·8%).

Alkyldicyandiamides. (b) *From* N-*Cyano*-S-*methyl*iso*thiourea Derivatives.*

N-*Cyano*-N′S-*dimethyl*iso*thiourea* (V; R = Me).—A mixture of methyl *iso*thiocyanate (7·3 g.) and sodium cyanamide (6·4 g.) in ethanol (150 c.c.) was stirred for 18 hours at laboratory temperature and then filtered from a little insoluble solid. Methyl iodide (28 g.) was added to the filtrate, and after ½ hour the crystalline suspension which formed was refluxed for ½ hour and cooled, and the product collected and washed with water and ethanol. After several recrystallisations from ethanol it had m. p. 196° (decomp.) (Found: C, 37·25; H, 5·7; N, 33·0; S, 24·8. Calc. for $C_4H_7N_3S$: C, 37·2; H, 5·45; N, 32·6; S, 24·8%). Hecht (*loc. cit.*) gave m. p. 194—195° for the same product. Similarly prepared were the homologues: N′-*ethyl*-, m. p. 161—163° (Found: C, 42·0; H, 5·95; N, 29·55; S, 22·4. $C_5H_9N_3S$ requires C, 41·95; H, 6·3; N, 29·35; S, 22·4%); N′-n-*propyl*-, m. p. 113° (Found: C, 45·9; H, 7·05; N, 27·15. $C_6H_{11}N_3S$ requires C, 45·85; H, 7·0; N, 26·75%); N′-iso*propyl*-, m. p. 117—119° (Found: C, 45·95; H, 6·9; N, 26·15; S, 20·9. $C_6H_{11}N_3S$ requires C, 45·85; H, 7·0; N, 26·75; S, 20·4%); N′-n-*butyl*-, m. p. 120° (Found: C, 48·75; H, 7·35; N, 24·75. $C_7H_{13}N_3S$ requires C, 49·1; H, 7·6; N, 24·55%); and N′-iso*butyl*-, m. p. 119—120° (Found: C, 49·4; H, 7·7; N, 24·75%).

The above *N*-cyano-*S*-methyl-*N'*-alkyl*iso*thioureas were converted into the corresponding *N*-alkyldicyandiamides by treatment with ammonia. The products in some experiments were viscous water-soluble oils which, however, were shown to consist mainly of the required substances by reaction with arylamine salts to give the expected N^1-aryl-N^5-alkyldiguanides in good yield. A typical experiment for the production of *iso*propyldicyandiamide was as follows. *N*-Cyano-*S*-methyl-*N'-iso*propyl*iso*thiourea (3 g.) and saturated alcoholic ammonia (50 c.c.) were heated in a sealed tube at 120° for 6 hours. The alcohol, excess of ammonia, and methylthiol were distilled off and the residual oil was dissolved in chloroform and filtered, and the solvent evaporated. Treatment of the residue with dioxan gave a solid which when recrystallised from the same solvent and dried in a vacuum at 70—75° had m. p. 101° and was identical with the product obtained by the dicyanimide route.

Aryldicyandiamides.

p-*Chlorophenyldicyandiamide* (II; X = *p*-Cl).—Cyanogen chloride was passed into calcium cyanamide (commercial fertiliser, 250 g.) and water (1 l.) cooled in ice–water, until the mixture was only faintly alkaline to litmus. The suspension was left overnight and filtered. A solution of *p*-chloroaniline (180 g.) in 3·5N-hydrochloric acid (410 c.c.) at 40° was added, and the mixture stirred for 24 hours at laboratory temperature. The precipitate which formed was collected, dissolved in 2N-sodium hydroxide (3 l.) at 60°, treated with charcoal, and filtered, and the filtrate made acid with hydrochloric acid. The pale cream crystalline precipitate of *p*-chlorophenyldicyandiamide when filtered off, washed with water, and dried (116 g.) had m. p. 197—200°. When recrystallised from ethanol it had m. p. 204—205° undepressed in admixture with material made as described in Part X (*loc. cit.*). Higher yields (*ca.* 80%) were obtained by interaction of sodium dicyanimide and *p*-chloroaniline hydrochloride either as above, at 60—70° for 6 hours, or at 85—90° for 2 hours.

p-*Iodophenyldicyandiamide* (II; X = *p*-I).—*p*-Iodoaniline hydrochloride (5·1 g.), sodium dicyanimide (1·8 g.), dioxan (30 c.c.), and water (100 c.c.) were stirred for 8 hours at 50—55°. The precipitate which formed was collected, washed with water, and dissolved in 2N-sodium hydroxide (60 c.c.) at 70—75°, the solution filtered, and the filtrate made acid with hydrochloric acid. The *product* recrystallised from wet ethanol, m. p. 222° (Found: C, 33·5; H, 2·7; N, 19·3. $C_8H_7N_4I$ requires C, 33·55; H, 2·45; N, 19·6%). Similar methods were used for the preparation of the corresponding phenyl- (m. p. 198—199°) and *p*-methoxyphenyl (m. p. 192°) dicyandiamides, and the identity of the products with those described in Part X established. For the preparation of *α-naphthyldicyandiamide* (VI; R = H, R' = α $C_{10}H_7$), naphthylamine (7·25 g.) in 2N-hydrochloric acid (25 c.c.) and ethanol (50 c.c.) was kept at 40° with sodium dicyanimide (5·5 g.) in water (20 c.c.). The insoluble oil that formed solidified on standing and when recrystallised from ethanol had m. p. 160—161° (Found: C, 68·4; H, 5·1; N, 26·1. $C_{12}H_{10}N_4$ requires C, 68·6; H, 4·8; N, 26·6%). NN-*Diphenyldicyandiamide* (VI; R = R' = Ph) was prepared in the same manner, using aqueous ethanol as solvent. The crude *product* obtained on adding water to the reaction mixture was freed from unchanged amine by treatment with toluene, and reprecipitated by addition of water to a filtered solution in acetone, and then gave colourless prisms from ethanol, m. p. 204—205° (Found: C, 70·8; H, 5·2; N, 24·2. $C_{14}H_{12}N_4$ requires C, 71·2; H, 5·1; N, 23·7%).

o-*Tolyldicyandiamide* (VI; R = H, R' = *o*-C_6H_4Me), colourless rhombs from ethanol, m. p. 131—132° (Found: C, 62·2; H, 6·05; N, 31·5. $C_9H_{10}N_4$ requires C, 62·2; H, 5·7; N, 32·2%), and *β-naphthyldicyandiamide* (VI; R = H, R' = β-$C_{10}H_7$), m. p. 248—249° (Found: C, 68·15; H, 4·5; N, 27·1. $C_{12}H_{10}N_4$ requires C, 68·6; H, 4·8; N, 26·6%), were prepared in the same way but using water only as the solvent. *N*-Methylaniline hydrochloride similarly gave N^1-*phenyl*-N^1-*methyldicyandiamide* (VI; R = Me, R' = Ph) which crystallised from toluene and had m. p. 135—136° (Found: C, 61·85; H, 6·0; N, 32·0. $C_9H_{10}N_4$ requires C, 62·2; H, 5·7; N, 32·2%). The preparation of the corresponding *p*-chlorophenyl derivative by methylation of *p*-chlorophenyldicyandiamide was described in Part X. Both substances are insoluble in dilute sodium hydroxide.

Diguanides.

N^1-p-*Chlorophenyl*-N^5-iso*propyldiguanide* (I; X = *p*-Cl, R = R' = H, R'' = Pr^β) ("*Paludrine*").—*p*-Chloroaniline hydrochloride (16·4 g.), *iso*propyldicyandiamide (12·9 g.), and water (50 c.c.) were refluxed for 4 hours. The cooled suspension was filtered, and the colourless crystals (yield, 22 g., 80%) when recrystallised from aqueous ethanol gave the diguanide hydrochloride, m. p. 243—244° undepressed in admixture with the hydrochloride made as described in Part X. The free base liberated by addition of sodium hydroxide to an aqueous-ethanolic suspension of the hydrochloride when crystallised from aqueous ethanol and air-dried at laboratory temperature formed colourless needles of the *monohydrate* and had m. p. 94—95° (Found: C, 48·75; H, 6·2; N, 25·4; Cl, 13·1. $C_{11}H_{16}N_5Cl,H_2O$ requires C, 48·6; H, 6·6; N, 25·8; Cl, 13·1%). Continued drying at 90—100° gave anhydrous material, m. p. 129°, identical with that obtained by recrystallisation from toluene (see Part X), but until dehydration was complete sintering at 104—106° gave evidence of an intermediate hydrate.

(a) N^1-p-*Substituted-phenyl*-N^5-iso*propyldiguanides*.—N^1-p-*Fluorophenyl*-N^5-iso*propyldiguanide* (I; X = *p*-F, R = R' = H, R'' = Pr^β). *p*-Fluoroaniline hydrochloride (6·7 g.), *iso*propyldicyandiamide (6·3 g.), and β-ethoxyethanol (40 c.c.) were refluxed for 5 minutes, the bulky precipitate which formed being broken up by shaking the mixture. After cooling, ethyl acetate (30 c.c.) was added and the suspension was filtered. The *hydrochloride* crystallised from water in colourless plates, m. p. 250—251° (Found: C, 48·7; H, 5·95; N, 24·9. $C_{11}H_{16}N_5F,HCl$ requires C, 48·25; H, 6·2; N, 25·6%) (5639). The product was identical with that made by the general method described in Part X (*loc. cit.*) by heating p-*fluorophenyldicyandiamide* (1·2 g.) [colourless prisms from water, m. p. 204—205° (Found: C, 53·9; H, 3·7; N, 32·2. $C_9H_7N_4F$ requires C, 53·9; H, 3·9; N, 31·5%)] and *iso*propylamine hydrochloride (1 g.) in nitrobenzene at 130—135° for 16 hours, extracting the resultant suspension with hot water, and crystallising the hydrochloride from the aqueous layer by chilling.

The following N^1-*p*-substituted-phenyl-N^5-*iso*propyldiguanide hydrochlorides were made similarly using the same solvent: N^1-p-*iodophenyl*- (I; X = *p*-I, R = R' = H, R'' = Pr^β), colourless needles

from water, m. p. 237—239° (Found: C, 34·9; H, 4·5; N, 18·1. $C_{11}H_{16}N_5I$,HCl requires C, 34·6; H, 4·45; N, 18·35%) (5744); N¹-p-*nitrophenyl*- (I; X = *p*-NO_2, R = R′ = H, R″ = Pr$^\beta$), yellow prismatic needles from water, m. p. 254° (decomp.) (Found: C, 44·35; H, 5·8; N, 27·8. $C_{11}H_{16}O_2N_6$,HCl requires C, 43·95; H, 5·65; N, 27·95%) (5858); N¹-p-*cyanophenyl*- (I; X = *p*-CN, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 239—241° (Found: C, 50·9; H, 5·85; N, 29·0. $C_{12}H_{16}N_6$,HCl requires C, 51·35; H, 6·05; N, 29·95%) (5859); and N¹-p-*tolyl*- (I; X = *p*-Me, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 240° (Found: C, 53·2; H, 7·45; N, 25·95. $C_{12}H_{19}N_5$,HCl requires C, 53·35; H, 7·4; N, 25·95%) (5909).

N¹-p-*Diphenylyl*-N⁵-iso*propyldiguanide* (I; X = *p*-Ph, R = R′ = H, R″ = Pr$^\beta$). *p*-Aminodiphenyl hydrochloride (5·5 g.), *iso*propyldicyandiamide (2·8 g.), and water (30 c.c.) were refluxed for 3 hours. The *hydrochloride* precipitated on cooling and recrystallised from aqueous ethanol in colourless needles, m. p. 253—254° (Found: C, 61·2; H, 6·2; N, 21·1. $C_{17}H_{21}N_5$,HCl requires C, 61·55; H, 6·55; N, 21·1%) (5983).

The following *N*¹-*p*-substituted-phenyl-*N*⁵-*iso*propyldiguanide hydrochlorides were made in a similar manner: N¹-p-*bromophenyl*- (I; X = *p*-Br, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 246° (Found: C, 39·55; H, 4·85; N, 20·2; Cl′, 10·8. $C_{11}H_{16}N_5Br$,HCl requires C, 39·45; H, 5·1; N, 20·95; Cl′, 10·6%) (5382); N¹-p-*ethoxyphenyl*- (I; X = *p*-OEt, R = R′ = H, R″ = Pr$^\beta$), colourless needles from water, m. p. 240° (Found: C, 51·85; H, 7·05; N, 23·55. $C_{13}H_{21}ON_5$,HCl requires C, 52·05; H, 7·35; N, 23·4%) (5568); N¹-p-*methylthiophenyl*- (I; X = *p*-SMe, R = R′ = H, R″ = Pr$^\beta$), colourless needles from water, m. p. 242—244° (Found: C, 47·85; H, 6·5; N, 23·6. $C_{12}H_{19}N_5S$,HCl requires C, 47·75; H, 6·65; N, 23·2%) (5739); and N¹-*phenyl*- (I; X = R = R′ = H, R″ = Pr$^\beta$), colourless plates from water, m. p. 235—236° (Found: C, 51·85; H, 6·95; N, 27·55. $C_{11}H_{17}N_5$,HCl requires C, 51·65; H, 7·0; N, 27·4%) (5771).

(b) N¹-o- *or* -m-*Substituted-phenyl*-N⁵-iso*propyldiguanides*.—The following hydrochlorides of this type were made by the methods described in (a). Reaction in water: N¹-o-*chlorophenyl*- (I; X = *o*-Cl, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 234—235° (Found: C, 45·6; H, 5·8; N, 24·3. $C_{11}H_{16}N_5Cl$,HCl requires C, 45·5; H, 5·85; N, 24·15%) (5741), and N¹-m-*chlorophenyl*- (I; X = *m*-Cl, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 232° (Found: C, 46·1; H, 6·15; N, 23·45. $C_{11}H_{16}N_5Cl$,HCl requires C, 45·5; H, 5·85; N, 24·15%) (5383). Reaction in β-ethoxyethanol: N¹-o-*tolyl*- (I; X = *o*-Me, R = R′ = H, R″ = Pr$^\beta$), colourless needles from water, m. p. 237° (Found: C, 53·15; H, 7·35; N, 25·6. $C_{12}H_{19}N_5$,HCl requires C, 53·45; H, 7·4; N, 25·95%) (5908); N¹-m-*tolyl*- (I; X = *m*-Me, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 216—217° (Found: C, 53·2; H, 7·6; N, 25·9. $C_{12}H_{19}N_5$,HCl requires C, 53·45; H, 7·4; N, 25·95%) (5910); and N¹-m-*cyanophenyl*- (I; X = *m*-CN, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 220—221° (Found: C, 51·5; H, 6·0; N, 29·8. $C_{12}H_{16}N_6$,HCl requires C, 51·35; H, 6·05; N, 29·95%) (5906); N¹-m-*carboxyphenyl*- (I; X = *m*-CO_2H, R = R′ = H, R″ = Pr$^\beta$), colourless prisms from water, m. p. 202—204° (Found: C, 48·65; H, 6·05; N, 22·9. $C_{12}H_{17}O_2N_5$,HCl requires C, 48·25, H, 5·7; N, 23·45%) (6655).

(c) N¹-*Substituted-phenyl*-N⁵: N⁵-*dialkyldiguanides*.—The following were obtained by the methods described in (a) above, using methyl*iso*propyldicyandiamide (VI; R = Me, R′ = Pr$^\beta$) and in one instance diethyldicyandiamide (VI; R = R′ = Et). Reaction in water: N¹-o-*chlorophenyl*-N⁵-*methyl*-N⁵-iso*propyldiguanide hydrochloride* (I; X = *o*-Cl, R = H, R′ = Me, R″ = Pr$^\beta$), colourless prisms from water, m. p. 227° (Found: C, 47·15; H, 6·1; N, 22·3. $C_{12}H_{18}N_5Cl$,HCl requires C, 47·35; H, 6·25; N, 23·0%) (5985); N¹-m-*chlorophenyl*-N⁵-*methyl*-N⁵-iso*propyldiguanide hydrochloride* (I; X = *m*-Cl, R = H, R′ = Me, R″ = Pr$^\beta$), colourless needles from water, m. p. 234° (Found: C, 47·15; H, 6·0; N, 23·0. $C_{12}H_{18}N_5Cl$,HCl requires C, 47·35; H, 6·25; N, 23·0%) (5984); N¹-p-*nitrophenyl*-N⁵-*methyl*-N⁵-iso*propyldiguanide hydrochloride* (I; X = *p*-NO_2, R = H, R′ = Me, R″ = Pr$^\beta$), yellow needles from water, m. p. 249° (Found: C, 45·75; H, 6·15; N, 26·75. $C_{12}H_{18}O_2N_6$,HCl requires C, 45·8; H, 6·05; N, 26·7%) (6001); and N¹-p-*cyanophenyl*-N⁵-*methyl*-N⁵-iso*propyldiguanide hydrochloride* (I; X = *p*-CN, R = H, R′ = Me, R″ = Pr$^\beta$), colourless needles from water, m. p. 252—253° (Found: C, 52·9; H, 6·25; N, 28·15. $C_{13}H_{18}N_6$,HCl requires C, 52·95; H, 6·45; N, 28·55%) (6002). Reaction in β-ethoxyethanol: *N*¹-*m*-chlorophenyl-*N*⁵: N⁵-diethyldiguanide (I; X = *m*-Cl, R = H, R′ = R″ = Et), colourless prisms from water, m. p. 226—227°; *hydriodide*, m. p. 218—219° (Found: C, 36·4; H, 4·9; N, 17·4. $C_{12}H_{18}N_5Cl$,HI requires C, 36·4; H, 4·8; N, 17·7%) (5944); and N¹-p-*iodophenyl*-N⁵-*methyl*-N⁵-iso*propyldiguanide hydrochloride* (I; X = *p*-I, R = H, R′ = Me, R″ = Pr$^\beta$), colourless needles from water, m. p. 236—237° (Found: C, 36·9; H, 4·9; N, 17·9. $C_{12}H_{18}N_5I$,HCl requires C, 36·5; H, 4·8; N, 17·7%) (6004).

(d) *Miscellaneous Diguanides*.—N¹-(6-*Bromo*-2-*naphthyl*)-N⁵-iso*propyldiguanide*. Prepared by method (a) above from 6-bromo-2-naphthylamine hydrochloride and *iso*propyldicyandiamide in β-ethoxyethanol, the *base* was isolated by warming the crude reaction precipitate in aqueous ethanolic sodium hydroxide, and formed colourless prisms from alcohol, m. p. 183° (Found: C, 52·0; H, 5·25; N, 19·9. $C_{15}H_{18}N_5Br$ requires C, 51·7; H, 5·15; N, 20·1%) (5437).

N¹-4-3′: 5′-*Dimethylphenoxyphenyl*-N⁵-iso*propyldiguanide* (I; X = -C_6H_4·O·$C_6H_3Me_2$-3 : 5, R = R′ = H, R″ = Pr$^\beta$). Prepared by refluxing 4-3′: 5′-dimethylphenoxyaniline hydrochloride and *iso*propyldicyandiamide in water, the *hydrochloride* formed colourless needles from water, m. p. 236—238° (Found: C, 60·6; H, 6·9; N, 18·7. $C_{19}H_{25}ON_5$,HCl requires C, 61·05; H, 6·95; N, 18·75%) (6101).

N¹-p-*Tolyl*-N⁵-*ethyldiguanide* (I; X = *p*-Me, R′ = H, R″ = Et). The *hydrochloride*, prepared from *p*-toluidine hydrochloride and ethyl dicyandiamide, crystallised from alcohol and had m. p. 222—223° (Found: C, 51·85; H, 7·0; N, 27·5. $C_{11}H_{17}N_5$,HCl requires C, 51·65; H, 7·05; N, 27·4%) (5567).

N¹-(6-*Quinolyl*)-N⁵-iso*propyldiguanide*. The *hydrochloride*, prepared from 6-aminoquinoline hydrochloride and *iso*propyldicyandiamide in boiling β-ethoxyethanol, crystallised from water, m. p. 241—242° (Found: C, 54·8; H, 6·1; N, 27·5. $C_{14}H_{18}N_6$,HCl requires C, 54·8; H, 6·2; N, 27·4%) (5738).

N¹-(8-*Chloro*-6-*quinolyl*)-N⁵-iso*propyldiguanide*. Prepared similarly from 8-chloro-6-aminoquinoline hydrochloride and *iso*propyldicyandiamide, the *hydrochloride* separated from water, m. p. 264—265° (Found: C, 49·1; H, 5·3; N, 24·2. $C_{14}H_{17}N_6Cl$,HCl requires C, 49·25; H, 5·3; N, 24·65%) (5907).

N^1-(6-*Methoxy*-8-*quinolyl*)-N^5-iso*propyldiguanide* (VII). Prepared similarly from *iso*propyldicyandiamide and 8-amino-6-methoxyquinoline hydrochloride in boiling β-ethoxyethanol, the *hydrochloride* crystallised from water and then had m. p. 237° (Found: C, 50·5; H, 6·6; N, 24·0. $C_{15}H_{20}ON_6,HCl,H_2O$ requires C, 50·8; H, 6·5; N, 23·7%) (5742).

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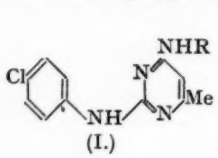
333. *Synthetic Antimalarials. Part XXIX. The Preparation of Some N^1-Aryl-N^2-alkyl-N^5-alkyl- and -dialkyl-diguanides.*

By A. F. CROWTHER, F. H. S. CURD, (MISS) D. N. RICHARDSON, and F. L. ROSE.

In order to extend the investigation of the relationship between chemical structure and antimalarial activity among N^1-aryl-N^5-alkyl- and -dialkyl-diguanides (Part X, Curd and Rose, *J.*, 1946, 729), a number of homologous N^2-alkyl derivatives have been prepared. Synthesis has been by two routes: (*a*) from mono- and di-alkylamines and N^1-aryl-N^2-alkyldicyandiamides, the latter prepared by condensation of an aryl *iso*thiocyanate with sodium cyanamide and treatment of the *S*-alkylated product with an alkylamine, and (*b*) by desulphurisation with mercuric oxide in the presence of an alkylamine of the *N*-aryl-*N'*-alkyl- (or dialkyl-)guanylthioureas resulting from the reaction of an aryl *iso*thiocyanate with a mono- or di-alkylguanidine. The use of ammonia in method (*b*) afforded the parent N^1-aryl-N^5-alkyl- and -dialkyl-diguanides, which were also formed by the action of ammonia alone on *N*-aryl-*N'*-alkyl- (or dialkyl-)guanyl-*S*-alkyl*iso*thioureas.

THE novel character of the diguanide type of antimalarial first described in Part X (Curd and Rose, *J.*, 1946, 729) called for a thorough investigation of the relationship between chemical structure and biological activity. The early preparations were mainly N^1-aryl-N^5-alkyl- and -dialkyl-diguanides of types (IV) and (V), although one or two derivatives carrying a methyl group on N^1 were also described. The influence of alkylation on N^2 was made the subject of early study, in particular because of the structural significance of this type in relation to the genesis of the diguanide drug type. Briefly, this arose from consideration of the activity of the *p*-chloroanilinodialkylaminoalkylaminopyrimidine types (I) and (II) in contrast to the inactivity of the isomeric 4-*p*-chloroanilino-6-dialkylaminoalkylamino-2-methylpyrimidines, first described in Part VIII (*J.*, 1946, 713), and the ultimate association of positive therapeutic activity with the presence in the first two molecular types of two linked amidine systems, each capable of independent tautomerism. It followed that this feature did not require the presence of a heterocyclic system *per se* but only of the functional skeleton (III), and although the analogous diguanide (IV; R = Cl, R' = $[CH_2]_2{\cdot}NEt_2$) modelled on this structure was without antimalarial activity, omission of part of the basic side chain led to active substances (V; R' and R'' = alkyl) and in due course to "Paludrine" (IV; R = Cl, R' = Pr^{β}). Closer inspection of the diguanide types (IV) and (V) and their pyrimidine prototype (I) shows the former to be derived by elimination of the carbon atoms in positions 5 and 6 of the pyrimidine ring and the introduction of an additional nitrogen atom. If, on the other hand, one or both of the carbon atoms in positions 5 and 6 of type (I) are retained, the corresponding diguanide is seen to be a N^1-aryl-N^2 : N^5-dialkyldiguanide [cf. (VI) and (VII; R'' = alkyl)].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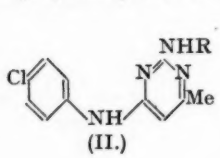
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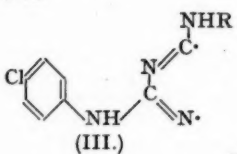
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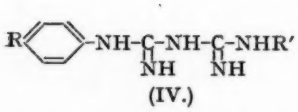
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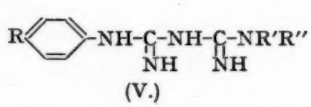
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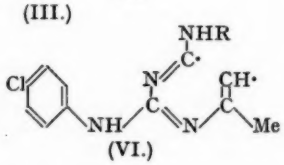
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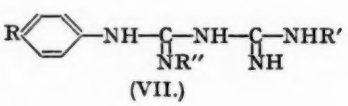
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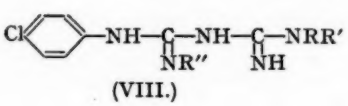
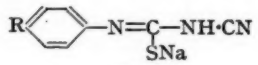

(VIII.)

The most remarkable property of the diguanide types (IV) and (V) was the prophylactic action they were found to exert against the different avian malarias (see Curd, Davey, and Rose, *Ann. Trop. Med. Parasit.*, 1945, **39**, 208). This prophylactic property moreover extends to human malaria (Fairley *et al.*, *Trans. Roy. Soc. Trop. Med. Hyg.*, 1946—47, **40**, 105, 621). Since the compounds of type (I) were inactive as prophylactics against avian malaria, it was clearly desirable to determine whether closer approximation of the diguanides of types (VII) and (VIII) to the skeleton (VI) of type (I) led to a loss of prophylactic activity, or whether the activity of the N^2-alkyl compounds on both the blood forms and early exo-erythrocytic forms of the avian malarias, relative to the parent types (III) and (IV), varied in the same way.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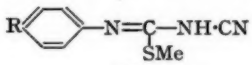
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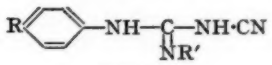
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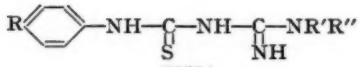
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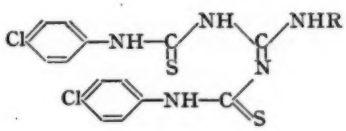
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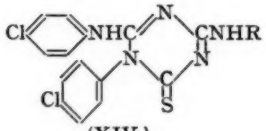
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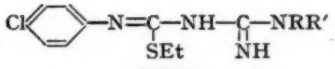
(XV.)

The diguanides of types (VII) and (VIII) have been synthesised by two main methods. The more obvious approach seemed to be an adaptation of the original method described in Part X for the preparation of types (IV) and (V), namely, condensation of a N^1-aryl-N^2-alkyldicyandiamide (XI) with a mono- or di-alkylamine. For the preparation of the necessary intermediates (XI) a modification of the method used by Wheeler and Jamieson (*J. Amer. Chem. Soc.*, 1903, **25**, 119) for the initial preparation of N^1-phenyldicyandiamide was employed. Thus *p*-chlorophenyl *iso*thiocyanate reacted with sodium cyanamide to give the *sodium* salt of N-*cyano*-N′-p-*chlorophenylthiourea* (IX; R = Cl) which with methyl iodide then gave N-*cyano*-N′-p-*chlorophenyl*-S-*methyl*isothiourea (X; R = Cl); treatment of this with aqueous or alcoholic methylamine at 50—60° yielded N¹-p-*chlorophenyl*-N²-*methyldicyandiamide* (XI; R = Cl, R′ = Me), and with ethylamine in a similar manner afforded N¹-p-*chlorophenyl*-N²-*ethyldicyandiamide* (XI; R = Cl, R′ = Et). Analogous reaction series, involving the steps (IX) ⟶ (X) ⟶ (XI), were used to prepare N¹-p-*bromophenyl*-N²-*methyldicyandiamide*, N¹-p-*iodophenyl*-N²-*methyldicyandiamide* and the corresponding N²-*ethyl* derivative. These various dicyandiamides of type (XI) were converted into diguanides either by direct fusion with a mono- or di-alkylamine hydrochloride or by heating the two reactants together in nitrobenzene at 130—135° (cf. Part XXVIII, preceding paper). In this way (XI; R = Cl, R′ = Me) and *iso*propylamine hydrochloride gave N^1-*p*-chlorophenyl-N^2-methyl-N^5-*iso*propyldiguanide (VII; R = Cl, R′ = Pr$^\beta$, R″ = Me) isolated as its *picrate*, while (XI; R = Cl, R′ = Et) afforded N¹-p-*chlorophenyl*-N²-*ethyl*-N⁵-iso*propyldiguanide hydrochloride*, both of which were identical with the products made by an alternative method described below. Numerous other examples of this type of synthesis are given in the experimental section. The dicyandiamides (XI; R = Cl, R′ = Me and Et) were also condensed with *p*-chloroaniline to give N¹ : N⁵-*di*-p-*chlorophenyl*-N²-*methyldiguanide* (VII; R = Cl, R′ = *p*-Cl·C$_6$H$_4$, R″ = Me) and the corresponding N²-*ethyldiguanide* (VII; R = Cl, R′ = *p*-Cl·C$_6$H$_4$, R″ = Et), but these compounds were devoid of antimalarial activity, and the type was not further investigated.

A method having advantages over that described above, since it has allowed facile variation of the N^2-alkyl group, involved conversion of the corresponding *N*-aryl-*N*′-alkyl- (and dialkyl-) guanylthioureas (XII) into diguanides of types (VII) and (VIII) by their reaction with alkylamines in presence of a desulphurising agent. The approach was specifically suggested by the method utilised in Part XXV (*J.*, 1948, 586) for the preparation of a number of 2-N^1-*p*-chlorophenyl-N^2-alkylguanidino-N^3-4-β-diethylaminoethylamino-6-methylpyrimidines, namely, the reaction of 2-*p*-chlorophenylthioureido-4-β-diethylaminoethylamino-6-methylpyrimidine with

an alkylamine in the presence of a desulphurising agent. There was also some indication in the literature that the desired analogous conversion might be effected in the diguanide series, Bamberger (*Ber.*, 1880, **13**, 1580) having shown that *N*-phenyl-*N'*-guanylthiourea (XII; R = R' = R'' = H) was convertible into phenyldiguanide by reaction with ammonia in the presence of a salt or oxide of silver or mercury. The same author and also Cramer (*Ber.*, 1901, **34**, 2602) found that the ammonia could be replaced by an arylamine with formation of a N^1 : N^2-diaryldiguanide. Further, Rathke and Oppenheim (*ibid.*, 1890, **23**, 1668) had demonstrated the conversion of *N*-phenyl-*N'*-(*NN'*-diphenylguanyl)thiourea into N^1 : N^2 : N^5-triphenyldiguanide by treatment with ammonia and silver nitrate.

For our investigations the synthesis of certain *N*-aryl-*N'*-alkyl- (and dialkyl-) guanylthioureas (XII) was required. Bamberger (*loc. cit.*; *Ber.*, 1881, **14**, 2638), Michael (*J. pr. Chem.*, 1894, **49**, 42), and Cramer (*loc. cit.*) have all described the preparation of *N*-phenyl-*N'*-guanylthiourea from guanidine and phenyl *iso*thiocyanate. Slotta, Tschesche, and Dressler (*Ber.*, 1930, **63**, 208; see also D.R.-P. 504,996) treated guanidine thiocyanate with acetone in which sodium had previously been dissolved, and brought the solution thus obtained into reaction with each one of a series of aryl *iso*thiocyanates. They were unable, however, to condense *iso*thiocyanates with mono- and tri-substituted guanidines. Methods of preparation of guanylthioureas other than by the condensation of a guanidine with an *iso*thiocyanate have been described by Slotta *et al.* (*loc. cit.*) who condensed the sodium salt of *N*-cyano-*N'*-phenylthiourea (IX; R = H) with aniline hydrochloride to give, in poor yield, *N*-phenyl-*N'*-phenylguanylthiourea, and by Fromm *et al.* (*Annalen*, 1908, **361**, 306, 319; 1912, **394**, 258) who caused 1-arylthiureas to react with arylamines to give, usually, mixtures of guanylthioureas.

Contrary to the statement of Slotta, Tschesche, and Dressler (*loc. cit.*), we have been able to effect the reaction of aryl *iso*thiocyanates not only with *NN*-dialkyl- but also with monoalkyl-guanidines to give guanylthioureas of the type (XII; R' = alkyl, R'' = H or alkyl). The conditions employed were essentially the same as those employed by Slotta and co-workers for many of their successful condensations, *i.e.*, by using the reaction mixture of sodium and acetone to liberate the guanidine from its salt and then effecting the condensation with the aryl *iso*thiocyanate in this mixture at about 30°. In this way *p*-chlorophenyl *iso*thiocyanate and *iso*propylguanidine gave N-p-*chlorophenyl*-N'-*isopropylguanylthiourea* (XII; R = Cl, R' = H, R'' = Pr^{β}), but condensations with some other monoalkylguanidines were more complex. Thus, in the preparation of N-p-*chlorophenyl*-N'-n-*butylguanylthiourea* (XII; R = Cl, R' = H, R'' = Bu^{α}) a by-product was isolated and analytical evidence suggested that it was 6-p-*chloroanilino*-4-n-*butylamino*-1-p-*chlorophenyl*-1 : 2-*dihydro*-1 : 3 : 5-*triazine*-2-*thione* (XIV; R = Bu^{α}). It was presumably formed by the condensation of 2 mols. of *p*-chlorophenyl *iso*thiocyanate with 1 mol. of *n*-butylguanidine followed by cyclisation of the resulting (XIII; R = Bu^{α}) to give (XIV; R = Bu^{α}) with elimination of hydrogen sulphide, and in this connection it may be noted that Slotta and co-workers (*loc. cit.*) observed that both allyl and *iso*amyl *iso*thiocyanate condensed with guanidine to give, in addition to the guanylthioureas, some of the corresponding dicondensation products or *NN'*-bis(alkylthiocarbamyl)guanidines. A product of similar type was formed in the reaction between *p*-chlorophenyl *iso*thiocyanate and methylguanidine and was presumed to be (XIV; R = Me), but none of the required *N*-*p*-chlorophenyl-*N'*-methylguanylthiourea could be isolated. However, *p*-chlorophenyl *iso*thiocyanate and ethylguanidine condensed normally to give N-p-*chlorophenyl*-N'-*ethylguanylthiourea* (XII; R = Cl, R' = H, R'' = Et) and other examples are described below.

The formation of *N*-aryl-*N'*-dialkylguanylthioureas was demonstrated by the reaction of phenyl *iso*thiocyanate with *NN*-dimethylguanidine to give N-*phenyl*-N'-(NN-*dimethylguanyl*)-*thiourea* (XII; R = H, R' = R'' = Me), by the preparation of the corresponding p-*chlorophenyl* derivative (XII; R = Cl, R' = R'' = Me), using *p*-chlorophenyl *iso*thiocyanate in place of phenyl *iso*thiocyanate, and by the condensation of *p*-chlorophenyl *iso*thiocyanate with *NN-cyclo*pentamethyleneguanidine to give (XII; R = Cl, R'R'' = $[CH_2]_5$).

As a model for the conversion of guanylthioureas of type (XII) into N^1-aryl-N^2-alkyl-N^5-mono- and di-alkyldiguanides, their conversion into diguanides of types (IV) and (V) was first investigated. This was achieved by stirring the guanylthiourea in alcoholic ammonia solution with mercuric oxide. In this way *N*-*p*-chlorophenyl-*N'*-*iso*propylguanylthiourea (XII; R = Cl, R' = H, R'' = Pr^{β}) was converted into N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (IV; R = Cl, R' = Pr^{β}) ("Paludrine") and *N*-*p*-chlorophenyl-*N'*-(*NN*-dimethylguanyl)thiourea (XII; R = Cl, R' = R'' = Me) into N^1-*p*-chlorophenyl-N^5 : N^5-dimethyldiguanide (V; R = Cl, R' = R'' = Me). Several other similar conversions are described in the experimental section.

In the same way, using an alkylamine in place of ammonia, it was found possible to prepare

N^1-aryl-N^2-alkyl-N^5-mono- or di-alkyldiguanides. Thus *N*-*p*-chlorophenyl-*N*′-*iso*propylguanylthiourea with methylamine gave N^1-*p*-chlorophenyl-N^2-methyl-N^5-*iso*propyldiguanide (VII; R = Cl, R′ = Pr$^\beta$, R″ = Me), and with ethylamine afforded N^1-*p*-chlorophenyl-N^2-ethyl-N^5-*iso*propyldiguanide (VII; R = Cl, R′ = Pr$^\beta$, R″ = Et), while N-p-*chlorophenyl*-N′-(NN-cyclo*pentamethyleneguanyl*)*thiourea* (XII; R = Cl, R′R″ = $[CH_2]_5$) was converted by the action of methylamine, under similar conditions, into N^1-p-*chlorophenyl*-N^5 : N^5-cyclo*pentamethylene*-N^2-*methyldiguanide* (VIII; RR′ = $[CH_2]_5$, R″ = Me) identical with the product of interaction of N^1-*p*-chlorophenyl-N^2-methyldicyandiamide with piperidine.

An alternative method for the conversion of guanylthioureas of type (XII) into diguanides of types (IV) and (V) is by *S*-alkylation to give the corresponding guanyl-*S*-alkyl*iso*thioureas followed by reaction of these with ammonia; a desulphurising agent is unnecessary. This was demonstrated by the conversion of (XII; R = Cl, R′ = H, R″ = Pr$^\beta$) and (XII; R = Cl, R′ = R″ = Me) into N-p-*chlorophenyl*-N′-iso*propylguanyl*-S-*ethyl*iso*thiourea hydrobromide* (as XV; R = H, R′ = Pr$^\beta$) and N-p-*chlorophenyl*-N′-(NN-*dimethylguanyl*)-S-*ethyl*iso*thiourea hydrobromide* (as XV; R = R′ = Me) respectively by the action of ethyl bromide, followed by heating of each of these latter compounds in turn with alcoholic ammonia at 100° in a closed vessel to give N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (IV; R = Cl, R′ = Pr$^\beta$) and N^1-*p*-chlorophenyl-N^5 : N^5-dimethyldiguanide (V; R = Cl, R′ = R″ = Me) as the respective products. The investigation of this method was prompted by the work of Slotta and Tschesche (*Ber.*, 1929, **62**, 1390, 1398) who prepared a number of N^1-substituted alkyldiguanides and alkylenebisdiguanides by interaction of guanyl-*S*-ethyl*iso*thiourea with substituted alkylamines and alkylenediamines, and described the preparation of N^1 : N^2-dimethyldiguanide and N^1 : N^1 : N^2-trimethyldiguanide by interaction of *N*-methyl-*N*′-guanyl-*S*-ethyl-*iso*thiourea and methylamine and dimethylamine respectively. U.S.P. 2,213,474 describes the preparation of N^1-dodecyldiguanide by an analogous method.

Many of the *N*-aryl-*N*′-alkyl- (and dialkyl-) guanylthioureas were tested by Dr. D. G. Davey for antimalarial activity against *P. gallinaceum* in chicks, but none was found to be active. The results of antimalarial tests on the N^2-alkyldiguanides of types (VII) and (VIII) will be discussed elsewhere.

Experimental.

N^1-*Aryl*-N^2-*alkyldicyandiamides and their Conversion into Diguanides.*

N-*Cyano*-N′-p-*chlorophenyl*-S-*methyl*iso*thiourea* (X; R = Cl).*—*p*-Chlorophenyl *iso*thiocyanate (50·75 g.) was added to a suspension of sodium cyanamide (19·2 g.) in alcohol (30 c.c.) with stirring. The sodium cyanamide gradually dissolved but before solution was complete the *sodium* salt of N-*cyano*-N′-p-*chlorophenylthiourea* began to separate. After 2 hours this was filtered off, washed with alcohol, and dried (Found: C, 41·1; H, 2·4. $C_8H_8N_3ClSNa$ requires C, 41·1; H, 2·1%) (yield, 36·2 g.). This compound (31·2 g.) was suspended in alcohol (200 c.c.) and methyl iodide (37·6 g.) added with rapid stirring. Heat was evolved and the product soon separated. When cold this was filtered off, washed with water and dried (yield, 26·2 g.). It crystallised from chlorobenzene and then had m. p 190—192° (Found: C, 47·9; H, 3·8; N, 18·5; Cl, 15·9; S, 14·9. $C_9H_8N_3ClS$ requires C, 47·9; H, 3·5; N, 18·6; Cl, 15·7; S, 14·2%).

N^1-p-*Chlorophenyl*-N^2-*methyldicyandiamide* (XI; R = Cl, R′ = Me).*—*N*-Cyano-*N*′-*p*-chlorophenyl-*S*-methyl*iso*thiourea (15 g.) was added to an alcoholic solution of methylamine (79·5 c.c. containing 4·14 g. of methylamine) and the mixture heated at 50° in a pressure bottle for 4 hours. The resulting clear solution was gradually diluted with water (75 c.c.); the *product* then crystallised out. It was filtered off (yield, 85%) and purified for analysis by crystallisation from water; colourless prisms, m. p. 169—171° (Found: C, 52·1; H, 4·6; N, 26·6; Cl, 16·8. $C_9H_9N_4Cl$ requires C, 51·8; H, 4·3; N, 26·8; Cl, 17·0%).

N^1-*p*-Chlorophenyl-N^2-methyldicyandiamide was also satisfactorily prepared by using aqueous methylamine in place of alcoholic methylamine.

N^1-p-*Chlorophenyl*-N^2-*ethyldicyandiamide* (XI; R = Cl, R′ = Et).*—Prepared similarly using alcoholic ethylamine in place of methylamine, this *compound* crystallised from water and then from benzene as colourless elongated prisms, m. p. 141—143° (Found: C, 54·2; H, 5·0; N, 25·6; Cl, 15·2. $C_{10}H_{11}N_4Cl$ requires C, 53·9; H, 4·9; N, 25·2; Cl, 15·95%).

N-*Cyano*-N′-p-*bromophenyl*-S-*methyl*iso*thiourea* (X; R = Br).—Sodium cyanamide (6·4 g.) was suspended in alcohol (100 c.c.) and *p*-bromophenyl *iso*thiocyanate (21·4 g.) (Dyson and George, *J.*, 1924, **125**, 1702) added. After 2 hours' stirring, methyl iodide (28·4 g.) was added and the stirring continued for a further 2 hours. The product was then filtered off, washed with water, dried, and crystallised from chlorobenzene, giving N-*cyano*-N′-p-*bromophenyl*-S-*methyl*iso*thiourea* as colourless thin prisms, m. p. 192—193° (decomp.) (Found: C, 40·3; H, 2·9; N, 15·8. $C_9H_8N_3Br$ requires C, 40·0; H, 3·0; N, 15·6%) (yield, 11·2 g.).

N-*Cyano*-N′-p-*iodophenyl*-S-*methyl*iso*thiourea* (X; R = I), prepared similarly from sodium cyanamide and *p*-iodophenyl *iso*thiocyanate [made from *p*-iodoaniline and thiocarbonyl chloride, m. p. 76—77° (Found: C, 32·3; H, 1·9; N, 5·7. Calc. for C_7H_4NIS: C, 32·6; H, 1·5; N, 5·4%); Losanitsch, *Ber.*, 1872, **5**, 156, gives m. p. 65°; Dyson and George, *loc. cit.*, give m. p. 63°], crystallised from chlorobenzene

* We are indebted to Dr. E. C. Owen for help with these preparations.

(a) N¹-*Phenyl*-N³-*alkyl*-N⁵-*alkyldiguanides* (Type VII).

Ref. No.	Substituent. R.	R′.	R″.	M. p.	Appearance.	Formula.	Analysis. Found, %. C.	H.	N.	Cl′.	Required, %. C.	H.	N.	Cl′.
5451	Cl	Et	Me	175—177°	Colourless prisms	$C_{11}H_{16}N_5Cl,HCl$	45·8	5·9	23·8	12·6	45·5	5·9	24·1	12·2
5495	Cl	Pr$^{\alpha}$	Me	177—179	Colourless flat prisms	$C_{12}H_{18}N_5Cl,HCl$	47·2	6·2	22·9	—	47·4	6·3	23·0	—
5471	Cl	Me	Et	174	Colourless prisms	$C_{11}H_{16}N_5Cl,HCl$	45·3	5·7	24·2	12·3	45·5	5·9	24·1	12·2
5453	Cl	Et	Et	175—177	Colourless rectangular prisms	$C_{12}H_{18}N_5Cl,HCl$	47·7	6·3	22·7	11·8	47·4	6·3	23·0	11·7
5496	Cl	Pr$^{\alpha}$	Et	175—177	Colourless prisms	$C_{13}H_{20}N_5Cl,HCl$	49·0	6·2	21·9	—	49·1	6·6	22·0	—
5511	Cl	CH_2Ph	Et	180—181	Colourless flat prisms	$C_{17}H_{20}N_5Cl,HCl$	55·6	5·7	19·6	—	55·7	5·7	19·1	—
5692	Br	Pr$^{\beta}$	Me	182—184	Colourless needles	$C_{12}H_{18}N_5Br,HCl$	41·3	5·8	20·3	—	41·3	5·5	20·1	—
6278	I	Pr$^{\beta}$	Me	212—214	Colourless elongated prisms	$C_{12}H_{18}N_5I,HCl$	36·5	4·8	17·0	9·3	36·4	4·8	17·7	9·0
6279	I	Pr$^{\beta}$	Et	219—220	Colourless elongated prisms	$C_{13}H_{20}N_5I,HCl$	37·9	5·0	17·1	—	38·1	5·1	17·1	—

(b) N¹-p-*Chlorophenyl*-N³-*alkyl*-N⁵-*disubstituted diguanides* (Type VIII).

Ref. No.	Substituent. R.	R′.	R″.	M. p.	Appearance.	Formula.	Analysis. Found, %. C.	H.	N.	Cl.	Required, %. C.	H.	N.	Cl.
5518	Et	Et	Me	165—167°	Colourless blunt-ended needles	$C_{13}H_{20}N_5Cl,C_2H_4O_2$	52·5	7·1	20·1	—	52·7	7·0	20·5	—
5472	$[CH_2]_5$		Me	204	Colourless prisms	$C_{14}H_{20}N_5Cl,HCl$	50·3	6·2	21·1	10·8 [Cl′]	50·9	6·4	21·2	10·75 [Cl′]
5520	Me	Pr$^{\beta}$	Et	184	Colourless blunt-ended needles	$C_{14}H_{22}N_5Cl,C_2H_4O_2$	54·0	7·6	19·1	—	54·0	7·3	19·7	—
5515	Et	Et	Et	173—174	Colourless blunt-ended needles	$C_{14}H_{22}N_5Cl,C_2H_4O_2$	54·0	6·9	19·4	—	54·0	7·3	19·7	—
5516	Me	Ph	Me	159—161	Colourless clumps of prisms	$C_{16}H_{18}N_5Cl,C_2H_4O_2$	57·2	5·6	18·6	8·9	57·5	5·8	18·6	9·0

as colourless prisms, m. p. 200—202° (decomp.) (Found : C, 34·2; H, 2·8; N, 13·3; S, 10·6. $C_9H_8N_3IS$ requires C, 34·1; H, 2·5; N, 13·2; S, 10·1%).

N^1-p-*Bromophenyl*-N^2-*methyldicyandiamide* (XI; R = Br, R′ = Me).—*N*-Cyano-*N*′-*p*-bromophenyl-*S*-methyl*iso*thiourea (10·8 g.), methylamine (12·4 g. of 21% aqueous solution), and alcohol (100 c.c.) were heated in a pressure bottle at 55—60° for 4 hours. After cooling, the contents were filtered and diluted with water to precipitate the *product* which crystallised from chlorobenzene as colourless flat prisms, m. p. 164—166° (Found : C, 42·9; H, 3·4; N, 21·5. $C_9H_9N_4Br$ requires C, 42·7; H, 3·6; N, 22·1%).

N^1-p-*Iodophenyl*-N^2-*methyldicyandiamide* (XI; R = I, R′ = Me), prepared similarly from *N*-cyano-*N*′-*p*-iodophenyl-*S*-methyl*iso*thiourea, crystallised from chlorobenzene as colourless flat prisms, m. p. 177—179° (Found : C, 36·3; H, 3·0; N, 18·3. $C_9H_9N_4I$ requires C, 36·0; H, 3·0; N, 18·7%).

N^1-p-*Iodophenyl*-N^2-*ethyldicyandiamide* (XI; R = I, R′ = Et), obtained in a similar manner from *N*-cyano-*N*′-*p*-iodophenyl-*S*-methyl*iso*thiourea and ethylamine, crystallised from benzene as colourless plates, m. p. 156—158° (Found : C, 38·3; H, 3·6; N, 17·6. $C_{10}H_{11}N_4I$ requires C, 38·2; H, 3·5; N, 17·8%).

N^1-p-*Chlorophenyl*-N^2-*methyl*-N^5-iso*propyldiguanide* (VII; R = Cl, R′ = Pr$^\beta$, R″ = Me).—N^1-*p*-Chlorophenyl-N^2-methyldicyandiamide (6·95 g.), *iso*propylamine hydrochloride (4·7 g.), and nitrobenzene (50 c.c.) were heated and stirred at 130—135° for 16 hours. The nitrobenzene solution was extracted with 2N-hydrochloric acid and the acid extract evaporated to dryness under reduced pressure. The residue was dissolved in water and the solution made alkaline with sodium hydroxide. The liberated base was extracted with ether and the ether extract dried (K_2CO_3). Evaporation left an oil which afforded, with picric acid in acetic acid solution, a *picrate*; yellow blunt ended needles from chlorobenzene, m. p. 163—164° (Found : C, 43·7; H, 4·4; N, 22·9. $C_{12}H_{18}N_5Cl,C_6H_3O_7N_3$ requires C, 43·5; H, 4·2; N, 22·6%).

N^1-p-*Chlorophenyl*-N^2-*ethyl*-N^5-iso*propyldiguanide* (VII; R = Cl, R′ = Pr$^\beta$, R″ = Et).—N^1-*p*-Chlorophenyl-N^2-ethyldicyandiamide (7·41 g.) and *iso*propylamine hydrochloride (4·7 g.) were heated in nitrobenzene (50 c.c.) at 130—135° for 16 hours. The solution was extracted with 2N-hydrochloric acid, and the acid solution evaporated to dryness. A solution of the residue in water was treated with decolourising carbon, filtered, and made slightly alkaline by addition of ammonia. The addition of salt; precipitated the *hydrochloride* which was collected, dried, and crystallised from alcohol–ethyl acetatet colourless plates, m. p. 174—176° (Found : C, 49·1; H, 6·7; N, 22·1; Cl′, 11·3. $C_{13}H_{20}N_5Cl,HCl$ requires C, 49·1; H, 6·6; N, 22·0; Cl′, 11·2%) (5335).

N^1-p-*Chlorophenyl*-N^2 : N^5-*dimethyl*-N^5-iso*propyldiguanide* (VIII; R = R″ = Me, R′ = Pr$^\beta$).—A mixture of N^1-*p*-chlorophenyl-N^2-methyldicyandiamide (6·95 g.), methyl*iso*propylamine hydrochloride (5·5 g.), and nitrobenzene (50 c.c.) was heated at 130—135° for 16 hours with stirring. The cooled mixture was extracted with 2N-hydrochloric acid, and the extract clarified with charcoal and made alkaline with sodium hydroxide. The precipitated base was extracted with ether, and the ethereal extract dried (Na_2SO_4) and evaporated. The residue was dissolved in acetone, and the solution made acid to litmus by addition of acetic acid. This precipitated the *acetate* which was collected and washed with acetone; it crystallised from acetone as colourless prisms, m. p. 192—194° (decomp.) (Found : C, 52·8; H, 6·9; N, 20·3. $C_{13}H_{20}N_5Cl,C_2H_4O_2$ requires C, 52·7; H, 7·0; N, 20·5%) (5523).

N^1-p-*Chlorophenyl*-N^2 : N^5-*dimethyldiguanide* (VII; R = Cl, R′ = R″ = Me).—N^1-*p*-Chlorophenyl-N^2-methyldicyandiamide (6·95 g.) and methylamine hydrochloride (3·37 g.) were stirred and heated at 130—135° for 16 hours. The resulting melt was dissolved in the minimum quantity of hot water, and the solution filtered and made alkaline with sodium hydroxide. The precipitated base was isolated by extraction with ether and evaporation of the dried (K_2CO_3) extract. The residual solid crystallised from light petroleum (b. p. 100—120°) to give N^1-p-*chlorophenyl*-N^2 : N^5-*dimethyldiguanide* as colourless prisms, m. p. 138° (Found : C, 50·4; H, 5·5; N, 28·6. $C_{10}H_{14}N_5Cl$ requires C, 50·1; H, 5·8; N, 29·2%). The *hydrochloride* separated from alcohol–ethyl acetate as colourless blunt-ended needles, m. p. 177—179° (Found : C, 42·9; H, 5·8; N, 25·0; Cl′, 13·3. $C_{10}H_{14}N_5Cl,HCl$ requires C, 43·5; H, 5·4; N, 25·4; Cl′, 12·9%) (5470).

By one or other of the above procedures, the N^1-aryl-N^2-alkyl-N^5-alkyl- and -N^5 : N^5-dialkyldiguanides described in the table were made.

N^1 : N^5-*Di*-p-*chlorophenyl*-N^2-*methyldiguanide* (VII; R = Cl, R′ = *p*-Cl·C$_6$H$_4$, R″ = Me).—N^1-*p*-Chlorophenyl-N^2-methyldicyandiamide (5·21 g.), *p*-chloroaniline hydrochloride (6·97 g.), dioxan (20 c.c.), and water (4 c.c.) were refluxed for 3½ hours. The mixture was cooled, diluted with water (175 c.c.), and allowed to stand overnight. The product was then collected, washed with water, dried, and crystallised from alcohol–ethyl acetate, giving the *hydrochloride* (5352) as clumps of colourless prisms, m. p. 239—241° (Found : C, 48·3; H, 4·3; N, 19·3; Cl, 28·1. $C_{15}H_{15}N_5Cl_2,HCl$ requires C, 48·3; H, 4·3; N, 18·8; Cl, 28·6%). This hydrochloride was converted into the *base* by dissolving it in alcohol, making the solution alkaline with sodium hydroxide, and diluting it with water. Isolated by extraction with chloroform and purified by crystallisation from light petroleum (b. p. 100—120°), it had m. p. 101—103° (Found : C, 54·3; H, 4·4; N, 20·1. $C_{15}H_{15}N_5Cl_2$ requires C, 53·6; H, 4·5; N, 20·8%).

N^1 : N^5-*Di*-p-*chlorophenyl*-N^2-*ethyldiguanide* (VII; R = Cl, R′ = *p*-Cl·C$_6$H$_4$, R″ = Et), prepared similarly, crystallised from light petroleum (b. p. 100—120°); m. p. 126—128° (Found : C, 55·1; H, 5·0; N, 19·7; Cl, 19·5. $C_{16}H_{17}N_5Cl_2$ requires C, 54·9; H, 4·9; N, 20·0; Cl, 20·3%). The *hydrochloride* crystallised from chlorobenzene as colourless prisms, m. p. 174—176° (Found : C, 49·5; H, 4·7; N, 18·1; Cl, 27·2. $C_{16}H_{17}N_5Cl_2,HCl$ requires C, 49·7; H, 4·7; N, 18·1; Cl, 27·6%) (5353).

N-*Aryl*-N′-*substituted-guanylthioureas*.

iso*Propylguanidine*.—*iso*Propylamine (118 g.) was added gradually, with stirring and cooling in an ice–salt bath, to a mixture of *S*-methyl*iso*thiourea sulphate (278 g.) and water (200 c.c.). The mixture was allowed to regain room temperature and left for 16 hours. It was then stirred at 30° for 3 hours and finally refluxed for 4·5 hours. The solution thus obtained was evaporated to dryness on the steam-bath and the residue crystallised from 95% alcohol, giving iso*propylguanidine sulphate* as colourless needles, m. p. 270—271° (Found : C, 31·7; H, 8·0. $C_4H_{11}N_3,0{\cdot}5H_2SO_4$ requires C, 32·0; H, 8·0%).

N-p-*Chlorophenyl*-N′-iso*propylguanylthiourea* (XII; R = Cl, R′ = H, R″ = Pr$^{\beta}$).—Sodium (0·75 g.) was gradually added with stirring and cooling to acetone (25 c.c.) which had previously been dried over anhydrous potassium carbonate and distilled over phosphoric oxide. *iso*Propylguanidine sulphate (4·85 g.) was then added and the mixture stirred for 1 hour at 30°. *p*-Chlorophenyl *iso*thiocyanate (4·2 g.) was added, and the stirring at 30° continued for 1 hour. Dilution of the mixture with water precipitated the *product* which was collected, washed with water, and crystallised, first from aqueous alcohol, and then from benzene; colourless flat prisms, m. p. 143° (Found: C, 49·2; H, 5·3; N, 20·8; Cl, 13·0. $C_{11}H_{15}N_4ClS$ requires C, 48·8; H, 5·5; N, 20·7; Cl, 13·1%) (5166).

N-p-*Chlorophenyl*-N′-iso*propylguanyl*-S-*ethyliso*thiourea (XV; R = H, R′ = Pr$^{\beta}$).—The above compound (2·7 g.) was dissolved in methanol (10 c.c.), and ethyl bromide (1·2 g.) added. The solution was refluxed for 2 hours and then evaporated to dryness on the steam-bath. The residual oil crystallised readily on cooling but could not be satisfactorily recrystallised. Analysis showed it to be substantially pure N-p-*chlorophenyl*-N′-iso*propylguanyl*-S-*ethyliso*thiourea *hydrobromide*, m. p. 146—148° (Found: C, 41·4; H, 5·4. $C_{13}H_{19}N_4ClS$,HBr requires C, 41·1; H, 5·3%).

N-p-*Chlorophenyl*-N′-n-*butylguanylthiourea* (XII; R = Cl, R′ = H, R″ = Bu^{n}).—*n*-Butylguanidine sulphate (35 g.) (m. p. 214—215°, prepared by interaction of *S*-methyl*iso*thiourea sulphate and *n*-butylamine; Davis and Elderfeld, *J. Amer. Chem. Soc.*, 1932, **54**, 1502, record m. p. 206°) was treated with sodium (3·74 g.) in dry acetone (125 c.c.), and then with *p*-chlorophenyl *iso*thiocyanate (21·2 g.) as described above in the case of the corresponding *iso*propyl compound. The crude product obtained on dilution of the reaction mixture with water was crystallised from a small amount of methanol. The product thus obtained was then crystallised from alcohol. A small crop of colourless needles separated. These were removed (see below) and the mother liquors diluted with water. The colourless plates thereby precipitated were collected, dried, and crystallised from benzene giving N-p-*chlorophenyl*-N′-n-*butylguanylthiourea* (5404), m. p. 114—116° (Found: C, 51·0; H, 5·4; N, 19·7; Cl, 12·0. $C_{12}H_{17}N_4ClS$ requires C, 50·6; H, 6·0; N, 19·7; Cl, 12·5%). The first substance obtained from the alcohol crystallisation was recrystallised from alcohol–2-ethoxyethanol and then had m. p. 182—183° (Found: C, 54·4; H, 4·4; N, 17·3; Cl, 16·8. $C_{19}H_{19}N_5Cl_2S$ requires C, 54·3; H, 4·5; N, 16·7; Cl, 16·9%). It was considered to be 6-p-*chloroanilino*-4-n-*butylamino*-1-p-*chlorophenyl*-1 : 2-*dihydro*-1 : 3 : 5-*triazine*-2-*thione* (XIV; R = Bu^{n}).

Condensation of p-*Chlorophenyl* iso*Thiocyanate with Methylguanidine*.—Methylguanidine sulphate (9·16 g.) was added to dry acetone (50 c.c.) containing dissolved sodium (1·5 g.) and the mixture stirred at 30° for 30 minutes. *p*-Chlorophenyl *iso*thiocyanate (8·48 g.) was then added at 10°, and the mixture stirred at 30—35° for 45 minutes and poured into water (300 c.c.). The reddish-brown oil which was precipitated was separated by decantation and stirred with aqueous alcohol. The crystalline material thus obtained was collected, washed with aqueous alcohol, dried, and crystallised from alcohol. The product formed hexagonal prisms, m. p. 233° (decomp.) (Found: C, 50·9; H, 3·6; N, 18·6; Cl, 19·2. $C_{16}H_{13}N_5Cl_2S$ requires C, 50·8; H, 3·4; N, 18·5; Cl, 18·8%), and appeared to be 6-p-*chloroanilino*-4-*methylamino*-1 : 2-*dihydro*-1 : 3 : 5-*triazine*-2-*thione* (XIV; R = Me). It was not desulphurised by alcoholic ammonia and mercuric oxide. The same substance was formed when methylguanidine was liberated from its sulphate by aqueous alcoholic potassium hydroxide and brought into reaction with *p*-chlorophenyl *iso*thiocyanate.

Ethylguanidine.—Ethylamine (68 g., 33% aqueous solution) was stirred with *S*-methyl*iso*thiourea sulphate (69·5 g.) at 30° for 6 hours and then at 90—100° for 16 hours. The mixture was evaporated to dryness on the steam-bath, and the crystalline residue broken up, and washed with alcohol. Crystallisation from aqueous alcohol gave *ethylguanidine sulphate* (yield, 62·5 g.) as colourless plates, m. p. 244—245° (Found: C, 26·7; H, 7·1. $C_3H_9N_3,0{\cdot}5H_2SO_4$ requires C, 26·5; H, 7·4%) (cf. Schenck and Kirchoff, *Z. physiol. Chem.*, 1926, **154**, 292, who prepared the hydriodide in a similar manner).

N-p-*Chlorophenyl*-N′-*ethylguanylthiourea* (XII; R = Cl, R′ = H, R″ = Et).—Ethylguanidine sulphate (39·4 g.) was treated with sodium (4·9 g.) dissolved in dry acetone (170 c.c.) and then with *p*-chlorophenyl *iso*thiocyanate (32·8 g.). The crude product, precipitated from the reaction mixture with water, was an oil but crystallised on trituration with benzene. It was then collected, washed with benzene, dried, and crystallised from alcohol, giving N-p-*chlorophenyl*-N′-*ethylguanylthiourea* as colourless needles, m. p. 134—135° (Found: C, 47·2; H, 5·4; N, 21·2; Cl, 14·1. $C_{10}H_{13}N_4ClS$ requires C, 46·8; H, 5·1; N, 21·8; Cl, 13·8%) (5465).

N-*Phenyl*-N′-(NN-*dimethylguanyl*)*thiourea* (XII; R = H, R′ = R″ = Me).—Sodium (3·0 g.) was dissolved in dry acetone (100 c.c.), *NN*-dimethylguanidine sulphate (20·4 g.) (Phillips and Clarke, *J. Amer. Chem. Soc.*, 1923, **45**, 1755; Davis and Elderfeld, *loc. cit.*) added, and the mixture stirred for 1 hour at 30°. Phenyl *iso*thiocyanate (13·4 g.) was then slowly added and stirring continued for 1 hour at 30°. The resulting mixture was poured into water (1 l.), and the precipitated product filtered off, washed with water, and crystallised from alcohol. Recrystallisation from alcohol gave N-*phenyl*-N′-(NN-*dimethylguanyl*)*thiourea*; colourless needles, m. p. 164° (Found: C, 53·6; H, 6·2. $C_{10}H_{14}N_4S$ requires C, 54·0; H, 6·3%).

N-p-*Chlorophenyl*-N′-(NN-*dimethylguanyl*)*thiourea* (XII; R = Cl, R′ = R″ = Me), prepared similarly from *NN*-dimethylguanidine sulphate and *p*-chlorophenyl *iso*thiocyanate, crystallised from methanol as colourless prisms, m. p. 160—161° (Found: C, 46·5; H, 5·1; N, 21·8. $C_{10}H_{13}N_4ClS$ requires C, 46·8; H, 5·1; N, 21·8%) (5385).

N-p-*Chlorophenyl*-N′-(NN-*dimethylguanyl*)-S-*ethyliso*thiourea (XV; R = R′ = Me).—The preceding compound (2·56 g.), ethyl bromide (1·19 g.), and methanol (10 c.c.) were gradually heated to boiling during 30 minutes. A further amount of ethyl bromide (0·76 g.) was added and the solution refluxed for 2 hours. Evaporation to dryness on the steam-bath gave a solid residue which on crystallisation from water afforded N-p-*chlorophenyl*-N′-(NN-*dimethylguanyl*)-S-*ethyliso*thiourea *hydrobromide* as colourless crystals, m. p. 204° (Found: C, 39·4; H, 5·2. $C_{12}H_{17}N_4ClS$,HBr requires C, 39·4; H, 4·9%) (5403).

NN-cyclo*Pentamethyleneguanidine*.—Piperidine (61 g.), *S*-methyl*iso*thiourea sulphate (100 g.), and water (80 c.c.) were stirred and the temperature gradually raised to 40° and kept there for 16 hours.

The mixture was then boiled for 8 hours under reflux. Subsequent evaporation to dryness on the steam-bath and lixiviation of the residue with alcohol (100 c.c.) gave the *sulphate* which crystallised from aqueous alcohol as colourless bipyramids, charring at 290° (Found: C, 41·1; H, 7·3; N, 23·8. $C_9H_{13}N_3,0{\cdot}5H_2SO_4$ requires C, 40·8; H, 7·9; N, 23·8%).

N-p-*Chlorophenyl*-N′-(NN-cyclo*pentamethyleneguanyl*)*thiourea* (XII; R = Cl, R′R″ = $[CH_2]_5$), prepared, in the manner described above, from NN-*cyclo*pentamethyleneguanidine sulphate and *p*-chlorophenyl *iso*thiocyanate, crystallised from xylene as almost colourless plates, m. p. 170° (Found: C, 52·8; H, 5·1; N, 18·8. $C_{12}H_{17}N_4ClS$ requires C, 52·6; H, 5·7; N, 18·9%) (5419).

N-p-*Bromophenyl*-N′-iso*propylguanylthiourea* (XII; R = Br, R′ = H, R″ = Pr$^\beta$).—Sodium (0·75 g.) was dissolved in dry acetone (25 c.c.), and finely divided *iso*propylguanidine sulphate (5·63 g.) added. After 1 hour's stirring *p*-bromophenyl *iso*thiocyanate (5·35 g.) was added with cooling, and the mixture then stirred for 1 hour at 30°. Dilution of the mixture with water (200 c.c.) gave a dark oil which hardened on being stirred. This product, isolated by decantation, was dissolved in alcohol (50 c.c.), and water gradually added with stirring to give a pale yellow solid. Crystallisation of this from benzene then gave N-p-*bromophenyl*-N′-iso*propylguanylthiourea* as small colourless plates, m. p. 146° (Found: C, 42·2; H, 4·6; N, 18·1. $C_{11}H_{15}N_4BrS$ requires C, 41·9; H, 4·8; N, 17·8%).

N-p-*Nitrophenyl*-N′-iso*propylguanylthiourea* (XII; R = NO_2, R′ = H, R″ = Pr$^\beta$), from *iso*propylguanidine sulphate and *p*-nitrophenyl *iso*thiocyanate [m. p. 110—111°, prepared by the method described by Dyson (*J.*, 1934, 176) for *o*-nitrophenyl *iso*thiocyanate; Jacobsen and Klein (*Ber.*, 1893, **26**, 2369) give m. p. 112—113°], formed lemon-yellow prisms from alcohol, m. p. 135° (Found: C, 46·8; H, 5·1; N, 25·1. $C_{11}H_{15}O_2N_5S$ requires C, 47·0; H, 5·3; N, 24·9%) (5772).

N-p-*Chlorophenyl*-N′-p-*chlorophenylguanylthiourea* (XII; R = Cl, R′ = H, R″ = *p*-Cl·C_6H_4).—(*a*) *p*-Chlorophenylguanidine (12 g.) (cf. Part XXV, this vol., p. 586) and *p*-chlorophenyl *iso*thiocyanate (12 g.) were heated in toluene (30 c.c.) at steam-bath temperature for 4 hours. The resulting mixture was filtered hot and the crystalline material which separated on cooling was collected, washed with benzene, and dried. Crystallisation from xylene gave the *compound* as colourless elongated prisms, m. p. 146—148° (Found: Cl, 20·3; S, 9·0. $C_{14}H_{12}N_4Cl_2S$ requires Cl, 20·9; S, 9·4%).

(*b*) *p*-Chlorophenyl *iso*thiocyanate (3 g.) and *p*-chlorophenylguanidine (3 g.) were heated on the steam-bath for 1 hour. The mixture gradually became homogeneous, and on cooling set to a yellowish transparent glass. Boiled with 2N-hydrochloric acid, this gave N-p-*chlorophenyl*-N′-p-*chlorophenylguanylthiourea hydrochloride* which crystallised from alcohol as clusters of colourless needles, m. p. 170—171° (Found: C, 44·9; H, 3·8; N, 14·9; Cl, 27·6. $C_{14}H_{13}N_4Cl_2S,HCl$ requires C, 44·7; H, 3·5; N, 14·9; Cl, 28·4%) (5373).

N^1-*Aryl*-N^5-*substituted-diguanides from* N-*aryl*-N′-*substituted-guanylthioureas.*

N^1-p-*Chlorophenyl*-N^5-iso*propyldiguanide* (IV; R = Cl, R′ = Pr$^\beta$).—(*a*) *N-p*-Chlorophenyl-*N′-iso*propylguanylthiourea (5 g.), mercuric oxide (5 g.), and methanolic ammonia (100 c.c. of 14%) were stirred for 3 hours at 35°. The mixture was then filtered and the residue washed with methanol. The filtrate and washings were evaporated on the steam-bath, leaving an oil which was dissolved in ethyl acetate (75 c.c.), and the solution filtered. Addition of acetic acid (2·5 c.c.) gave a copious white precipitate on standing, which was collected, dried, and identified as *N*^{1}-*p*-chlorophenyl-*N*^{5}-*iso*propyldiguanide acetate (cf. Part X, *J.*, 1946, 729), m. p. and mixed m. p. 185°. Conversion of a portion of the acetate into the base by dissolving it in water and making the solution alkaline with ammonia, gave a product which crystallised from toluene and then had m. p. and mixed m. p. 130—131°.

(*b*) *N-p*-Chlorophenyl-*N′-iso*propylguanyl-*S*-ethyl*iso*thiourea hydrobromide (1 g.) was heated with saturated alcoholic ammonia (7 c.c.) in a sealed tube at 100° for 1 hour. On opening the tube there was a strong odour of ethylthiol. The contents of the tube were evaporated to dryness, the residue extracted with 2N-hydrochloric acid, and the extract, after treatment with decolourising carbon, made alkaline with sodium hydroxide and extracted with benzene. After evaporation of the dried benzene extract, the residue was dissolved in ethyl acetate and treated with acetic acid to give *N*^{1}-*p*-chlorophenyl-*N*^{5}-*iso*propyldiguanide acetate, m. p. and mixed m. p. 185°.

N^1-p-*Chlorophenyl*-N^5-*ethyldiguanide* (IV; R = Cl, R′ = Et).—*N-p*-Chlorophenyl-*N′*-ethylguanylthiourea (1 g.), mercuric oxide (2 g.), and alcoholic ammonia (20 c.c.) were kept at 30—35° for 22 hours, and the mixture then worked up as described under (*a*) above to give *N*^{1}-*p*-chlorophenyl-*N*^{5}-ethyldiguanide acetate, m. p. 161—162° undepressed on admixture with an authentic sample (Part X, *loc. cit.*).

N^1-p-*Chlorophenyl*-N^5-n-*butyldiguanide* (IV; R = Cl, R′ = Bu^n).—*N-p*-Chlorophenyl-*N′-n*-butylguanylthiourea (1·14 g.) was treated in a similar manner with mercuric oxide (2·28 g.) and alcoholic ammonia (20 c.c.), and the mixture was then filtered and evaporated to dryness. The residue was dissolved in alcoholic hydrogen chloride and excess of ethyl acetate added. The precipitated hydrochloride crystallised from water to give *N*^{1}-*p*-chlorophenyl-*N*^{5}-*n*-butyldiguanide hydrochloride, m. p. 208° undepressed by admixture with an authentic specimen (Part X, *loc. cit.*).

N^1-p-*Bromophenyl*-N^5-iso*propyldiguanide* (IV; R = Br, R′ = Pr$^\beta$).—*N-p*-Bromophenyl-*N′-iso*propylguanylthiourea (1·5 g.), mercuric oxide (3·0 g.), and alcoholic ammonia (25 c.c.) were stirred at 30—40° for 17 hours. The mixture was then made acid with 2N-hydrochloric acid, treated with sodium sulphide, and filtered. The filtrate was basified with sodium hydroxide and extracted with benzene. Evaporation of the dried benzene extract, dissolution of the residue in 2N-hydrochloric acid, and filtration, gave a solution from which the hydrochloride was precipitated by neutralisation with ammonia and addition of salt. Crystallisation of the dried crude hydrochloride from alcohol–ethyl acetate gave colourless rosettes of needles, m. p. and mixed m. p. 245—246° (Curd *et al.*, preceding paper, give m. p. 246°).

N^1-*Phenyl*-N^5 : N^5-*dimethyldiguanide* (V; R = H, R′ = R″ = Me).—*N*-Phenyl-*N′*-(*NN*-dimethylguanyl)thiourea (10 g.), mercuric oxide (15 g.), and alcoholic ammonia (70 c.c. of 6%) were stirred at 30—40° for 17 hours. The mixture was then filtered, and the black residue washed with alcohol. The filtrate and washings were combined and evaporated to dryness, the residue dissolved in 2N-hydrochloric

acid, and the solution filtered and neutralised (to litmus) with ammonia. Addition of salt precipitated a solid which was collected, washed with brine, dried, and crystallised from alcohol–ethyl acetate to give N^1-*phenyl*-N^5 : N^5-*dimethyldiguanide hydrochloride* as colourless feathery needles, m. p. 240° (Found : C, 49·6; H, 6·4; N, 28·5. $C_{10}H_{15}N_5$,HCl requires C, 49·7; H, 6·6; N, 29·0%).

N^1-p-*Chlorophenyl*-N^5 : N^5-*dimethyldiguanide* (V; R = Cl, R′ = R″ = Me).—(*a*) A mixture of *N*-*p*-chlorophenyl-*N*′-(*NN*-dimethylguanyl)thiourea (1·28 g.), mercuric oxide (2·16 g.), and alcoholic ammonia (20 c.c.) were treated as described above for *N*^{1}-*p*-chlorophenyl-*N*^{5}-*iso*propyldiguanide, to give the base which crystallised from toluene as colourless needles, m. p. 168—169°, identical with an authentic specimen made from *p*-chlorophenyldicyandiamide and dimethylamine (Part X).

(*b*) *N*-*p*-Chlorophenyl-*N*′-(*NN*-dimethylguanyl)-*S*-ethyl*iso*thiourea hydrobromide (1 g.) was heated with alcoholic ammonia (5 c.c.) in a sealed tube at 100° for 1 hour. After cooling, the contents of the tube were evaporated to dryness and the residue extracted with 2N-hydrochloric acid. The acid extract was filtered and then poured into dilute sodium hydroxide solution. The precipitated solid was filtered off, washed with water, dried, and crystallised from toluene, giving *N*^{1}-*p*-chlorophenyl-*N*^{5} : *N*^{5}-dimethyldiguanide, m. p. and mixed m. p. 169°.

N^1-*p*-Chlorophenyl-*N*^{5} : *N*^{5}-*cyclo*pentamethylenediguanide (V; R = Cl, R′R″ = $[CH_2]_5$), prepared by the action of alcoholic ammonia (20 c.c.) and mercuric oxide (2 g.) on *N*-*p*-chlorophenyl-*N*′-(*NN*-*cyclo*pentamethyleneguanyl)thiourea (1 g.), crystallised from xylene as colourless rods, m. p. 190—191°, undepressed on admixture with material made by the method described in Part X.

N^1 : N^5-*Di*-p-*chlorophenyldiguanide* (IV; R = Cl, R′ = *p*-Cl·C_6H_4).—A mixture of *N*-*p*-chlorophenyl-*N*′-(*p*-chlorophenylguanyl)thiourea (4 g.), mercuric oxide (4 g.), and alcoholic ammonia (25 c.c.) was stirred at 30—35° for 22 hours. Evaporation of the filtered reaction mixture gave the base which was dissolved in alcoholic hydrogen chloride and the solution evaporated to dryness. The residual *hydrochloride* crystallised from alcohol as colourless needles (yield, 1·2 g.), m. p. 250° (Found : C, 46·9; H, 3·7; N, 19·8; Cl, 29·8. $C_{14}H_{13}N_5Cl_2$,HCl requires C, 46·9; H, 3·9; N, 19·5; Cl, 29·7%) (5367).

N^1-*Aryl*-N^3-*alkyl*-N^5-*alkyl- and -dialkyl-diguanides from* N-*Aryl*-N′-*alkyl- and -dialkyl-guanylthioureas.*

N^1-p-*Chlorophenyl*-N^3-*methyl*-N^5-iso*propyldiguanide* (VII; R = Cl, R′ = Pr$^\beta$, R″ = Me).—*N*-*p*-Chlorophenyl-*N*′-*iso*propylguanylthiourea (4 g.), mercuric oxide (4 g.), aqueous methylamine (25 c.c. of 21%), alcohol (35 c.c.), and 2-ethoxyethanol (10 c.c.) were stirred at 50° for 20 hours. The mixture was then filtered and the filtrate evaporated to dryness. The residue was extracted with 2N-hydrochloric acid, the extract added to sodium hydroxide, and the precipitated base filtered off, washed with water, and dried. The base could not be crystallised satisfactorily, but its identity with the product made by condensation of *N*^{1}-*p*-chlorophenyl-*N*^{3}-methyldicyandiamide with *iso*propylamine (see above) was demonstrated by conversion into the picrate, m. p. and mixed m. p. 163—164°. The *dihydrochloride* (5363), prepared by dissolving the base in alcohol and making the solution faintly acid to Congo-red with alcoholic hydrogen chloride, followed by precipitation with ethyl acetate, separated from alcohol–ethyl acetate as a colourless microcrystalline powder, m. p. 166—168° (Found : C, 40·1; H, 5·9; N, 20·4; Cl, 29·4. $C_{12}H_{18}N_5Cl,2HCl,H_2O$ requires C, 40·2; H, 6·1; N, 19·5; Cl, 29·7%).

N^1-p-*Chlorophenyl*-N^3-*ethyl*-N^5-iso*propyldiguanide* (VII; R = Cl, R′ = Pr$^\beta$, R″ = Et).—*N*-*p*-Chlorophenyl-N′-*iso*propylguanylthiourea (4 g.), mercuric oxide (4 g.), aqueous ethylamine (20 c.c. of 33%), and alcohol (35 c.c.) were stirred at 50—55° for 4 hours. 2N-Hydrochloric acid was added followed by sodium sulphide, and the mixture filtered. The filtrate was added to sodium hydroxide, and the crude base thereby precipitated was collected, dried, and dissolved in alcoholic hydrogen chloride. Addition of ethyl acetate precipitated the hydrochloride which was filtered off, dried, and crystallised from alcohol–ethyl acetate to give the same hydrochloride as described above, m. p. and mixed m. p. 174—176°.

N^1-p-*Chlorophenyl*-N^3 : N^5-*diiso*propyldiguanide (VII; R = Cl, R′ = R″ = Pr$^\beta$).—*N*-*p*-Chlorophenyl-*N*′-*iso*propylguanylthiourea (5 g.), mercuric oxide (5 g.), and *iso*propylamine (5 c.c.) were kept in alcohol (25 c.c.) at 30—35° for 6 hours and worked up as described above to give the *hydrochloride* which crystallised from alcohol–ethyl acetate as colourless feathery needles, m. p. 200—202° (Found : C, 50·2; H, 7·0; N, 20·9; Cl, 21·9. $C_{14}H_{22}N_5Cl$,HCl requires C, 50·6; H, 6·9; N, 21·1; Cl, 21·4%) (5351).

N^1-p-*Chlorophenyl*-N^5-iso*propyl*-N^3-n-*butyldiguanide* (VII; R = Cl, R′ = Pr$^\beta$, R″ = Bu$^\alpha$).—Prepared similarly using *n*-butylamine in place of *iso*propylamine, this formed a *hydrochloride* which crystallised from alcohol–ethyl acetate as colourless prisms, m. p. 182—183° (Found : C, 51·8; H, 7·2; N, 20·8; Cl, 20·9. $C_{15}H_{24}N_5Cl$,HCl requires C, 52·0; H, 7·2; N, 20·2; Cl, 20·5%) (5350).

N^1-p-*Chlorophenyl*-N^5-*ethyl*-N^3-n-*propyldiguanide* (VII; R = Cl, R′ = Et, R″ = Pr$^\alpha$).—*N*-*p*-Chlorophenyl-*N*′-ethylguanylthiourea (6 g.), mercuric oxide (12 g.), *n*-propylamine (6 c.c.), and alcohol (30 c.c.) were heated at 30—40° for 18 hours with stirring. 2N-Hydrochloric acid and sodium sulphide were added to precipitate all the mercury in the form of sulphide. The mixture was filtered, and the filtrate made alkaline with sodium hydroxide and extracted with benzene. Removal of the solvent from the extract left the base which was dissolved in 2N-hydrochloric acid, and the solution neutralised with ammonia. Addition of salt precipitated a solid which was collected, washed with water, and dried. Crystallisation of this from alcohol–ethyl acetate afforded the *hydrochloride* as colourless needles, m. p. 136° (Found : C, 48·8; H, 6·1; N, 22·5; Cl, 22·1. $C_{13}H_{20}N_5Cl$,HCl requires C, 49·1; H, 6·6; N, 22·0; Cl, 22·3%) (5541).

The following were made in an analogous manner : N^1-p-*chlorophenyl*-N^5-*ethyl*-N^3-iso*propyldiguanide hydrochloride* (as VII; R = Cl, R′ = Et, R″ = Pr$^\beta$), minute colourless prisms, m. p. 178—179°, from alcohol–ethyl acetate (Found : C, 48·9; H, 6·8; N, 21·9; Cl, 21·9. $C_{12}H_{20}N_5Cl$,HCl requires C, 49·1; H, 6·6; N, 22·0; Cl, 22·3%) (5576); N^1-p-*chlorophenyl*-N^5-*ethyl*-N^3-n-*butyldiguanide hydrochloride* (as VII; R = Cl, R′ = Et, R″ = Bu$^\alpha$), small colourless needles, m. p. 152°, from alcohol–ethyl acetate (Found : C, 50·4; H, 6·6; N, 20·6; Cl, 21·4. $C_{14}H_{22}N_5Cl$,HCl requires C, 50·6; H, 6·9; N, 21·1; Cl, 21·4%) (5577); N^1-p-*chlorophenyl*-N^5-*ethyl*-N^3-iso*butyldiguanide hydrochloride* (as VII; R = Cl, R′ = Et, R″ = Bu$^\beta$), hexagonal prisms, m. p. 175°, from alcohol–ethyl acetate (Found : C, 50·9; H, 7·2; N, 20·9; Cl, 21·2. $C_{14}H_{22}N_5Cl$,HCl requires C, 50·6; H, 6·9; N, 21·1; Cl, 21·4%) (5601).

N¹-p-*Chlorophenyl*-N²-*methyl*-N⁵-n-*butyldiguanide* (VII; R = Cl, R′ = Bu^{n}, R″ = Me).—*N*-*p*-Chlorophenyl-*N*′-*n*-butylguanylthiourea (6 g.) in alcohol (120 c.c.) was treated with aqueous methylamine (30 c.c. of 21%) and mercuric oxide (12 g.), and the reaction mixture worked up as described above for 5541. The *hydrochloride* crystallised from alcohol–ethyl acetate as colourless prisms, m. p. 160° (Found: C, 48·6; H, 6·3; N, 21·5; Cl, 22·3. $C_{13}H_{20}N_5Cl,HCl$ requires C, 49·1; H, 6·6; N, 22·0; Cl, 22·3%) (5600).

The following were made in an analogous manner: N¹-p-*chlorophenyl*-N²-*ethyl*-N⁵-n-*butyldiguanide hydrochloride* (as VII; R = Cl, R′ = Bu^{n}, R″ = Et), colourless prisms, m. p. 177°, from water (Found: C, 50·6; H, 6·8; N, 21·2; Cl, 21·2. $C_{14}H_{22}N_5Cl,2HCl$ requires C, 50·5; H, 6·9; N, 21·1; Cl, 21·4%) (5542); *N*¹-*p*-bromophenyl-*N*²-methyl-*N*⁵-*iso*propyldiguanide hydrochloride (as VII; R = Br, R′ = Pr$^{\beta}$, R″ = Me), from *N*-*p*-bromophenyl-*N*′-*iso*propylguanylthiourea and methylamine, m. p. 182—184° undepressed in admixture with material prepared from *N*¹-*p*-bromophenyl-*N*²-methyldicyandiamide and *iso*propylamine (see above); N¹-p-*bromophenyl*-N²-*ethyl*-N⁵-iso*propyldiguanide hydrochloride* (as VII; R = Br, R′ = Pr$^{\beta}$, R″ = Et), small colourless needles, m. p. 180°, from alcohol–ethyl acetate (Found: C, 42·5; H, 5·6. $C_{13}H_{20}N_5Br,HCl$ requires C, 43·0; H, 5·8%) (5693).

N¹-p-*Chlorophenyl*-N⁵: N⁵-cyclo*pentamethylene*-N²-*methyldiguanide* (VIII; R and R′ = $[CH_2]_5$, R″ = Me).—*N*-*p*-Chlorophenyl-*N*′-(*NN*-*cyclo*pentamethyleneguanyl)thiourea (5 g.), mercuric oxide (10 g.), aqueous methylamine (25 c.c. of 21%), and alcohol (75 c.c.) were stirred at 30—35° for 22 hours. The filtered reaction mixture was evaporated to dryness on the steam-bath, and the residue crystallised from aqueous alcohol, giving the *base* as colourless prisms, m. p. 142—143° (Found: C, 57·2; H, 6·8; Cl, 12·0. $C_{14}H_{20}N_5Cl$ requires C, 57·2; H, 6·8; Cl, 12·1%). The hydrochloride crystallised from alcohol–ethyl acetate as colourless rods, m. p. 204° either alone or admixed with material made from *N*¹-*p*-chlorophenyl-*N*²-methyldicyandiamide and piperidine (see Table).

N¹-p-*Chlorophenyl*-N⁵: N⁵-cyclo*pentamethylene*-N²-*ethyldiguanide* (VIII; R and R′ = $[CH_2]_5$, R″ = Et), prepared similarly using ethylamine in place of methylamine, crystallised from aqueous alcohol as long colourless needles, m. p. 121—123° (Found: C, 58·0; H, 6·9. $C_{15}H_{22}N_5Cl$ requires C, 58·5; H, 7·2%) (5473).

N¹-p-*Chlorophenyl*-N²: N⁵-*trimethyldiguanide* (VIII; R = R′ = R″ = Me).—*N*-*p*-Chlorophenyl-*N*′-(*NN*-dimethylguanyl)thiourea (5 g.) in alcohol (75 c.c.) was treated with mercuric oxide (10 g.) and aqueous methylamine (25 c.c. of 21%) at 30—35° for 22 hours. The filtered mixture was evaporated on the steam-bath, the residual crude base dissolved in ethyl acetate, and the solution neutralised with acetic acid to give the acetate. This was reconverted into the base by dissolution in water and treatment with sodium hydroxide. The base was then extracted with benzene, and the solution dried (K_2CO_3) and evaporated. The residue was then again dissolved in ethyl acetate and treated with acetic acid to give the *acetate* which crystallised from alcohol–acetone as colourless needles, m. p. 172° (Found: C, 49·2; H, 6·4; N, 22·3; Cl, 11·2. $C_{11}H_{16}N_5Cl,C_2H_4O_2$ requires C, 49·8; H, 6·4; N, 22·3; Cl, 11·3%) (5462).

N¹-p-*Chlorophenyl*-N⁵: N⁵-*dimethyl*-N²-*ethyldiguanide* (VIII; R = R′ = Me, R″ = Et).—Similarly prepared using ethylamine in place of methylamine, this formed an *acetate* which crystallised from acetone–alcohol as colourless needles, m. p. 185° (Found: C, 51·6; H, 6·6; N, 10·5. $C_{12}H_{18}N_5Cl,C_2H_4O_2$ requires C, 51·3; H, 6·7; Cl, 10·8%) (5454).

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334. *Synthetic Antimalarials. Part XXX. Some* N¹-*Aryl*-N⁴: N⁵-*Dialkyldiguanides and Observations on the Conversion of Guanylthioureas into Diguanides.*

By S. BIRTWELL, F. H. S. CURD, J. A. HENDRY, and F. L. ROSE.

A series of *N*¹-*p*-chlorophenyl-*N*⁴: *N*⁵-dialkyldiguanides (III; R and R′ = alkyl) has been synthesised, using two general methods, in order to determine the effect on antimalarial activity of introducing an alkyl group on *N*⁴ of the *N*¹-*p*-chlorophenyl-*N*⁵-alkyldiguanides described in Part X (*J.*, 1946, 729). The first method involves the reaction of *N*-cyano-*S*-methyl-*N*′-alkyl*iso*thioureas with alkylamines to give *N*¹: *N*³-dialkyldicyandiamides which are then condensed with *p*-chloroaniline. In the second method the alkylamine is caused to react, in presence of a desulphurising agent, with *N*-*p*-chlorophenylguanyl-*N*′-alkylthioureas obtained from *p*-chlorophenylguanidine and alkyl *iso*thiocyanates: the use of ammonia in place of the alkylamine gives the parent diguanide type (III; R = alkyl, R′ = H).

The observation that *N*-arylguanyl- and *N*-alkylguanyl-thioureas with amines and desulphurising agents such as mercuric oxide give the corresponding dicyandiamides, diguanides being formed in reasonable yield only under conditions normally required for the reaction of the latter with amines, suggests that the conversion of *N*-arylguanyl-*N*′-alkylthioureas into diguanides may proceed analogously through the intermediate production of carbodi-imides (X).

The *S*-alkyl derivatives of the several types of guanylthioureas referred to above also yield diguanides under certain conditions, the intervention of a desulphurising agent being then unnecessary.

IN connection with the study being made in these laboratories of the effect of changes of chemical constitution on antimalarial activity among derivatives of diguanide, a number of *N*¹-*p*-chloro-

phenyl-N^4 : N^5-dialkyldiguanides (III; R and R′ = alkyl) has been made. It is the purpose of the present paper to record the synthetic methods that have been used. Compounds of type (III) can be considered as derived from the earlier pyrimidine type (II) (*J.*, 1946, 370, 720) in the same way that the N^1-aryl-N^3 : N^5-dialkyldiguanides (IV; R′ and R″ = alkyl), described in Part XXIX (this vol., p. 1636) were evolved from the original pyrimidine type (I). Fission of the pyrimidine ring of the type (II) along the line *a* leaves a skeleton (V) which is incorporated in the diguanides of type (III; R and R′ = alkyl).

A very convenient method for the preparation of diguanides of type (III) was found to be the condensation of *p*-chloroaniline with N^1 : N^3-dialkyldicyandiamides (VII; R and R′ = alkyl). This type of disubstituted dicyandiamide, hitherto unknown, has been synthesised by condensing *N*-cyano-*S*-methyl-*N*′-alkyl*iso*thioureas (VI; R = alkyl) with alkylamines. The synthesis is analogous to that initially described and used in Part XXVIII (this vol., p. 1630) for the preparation of N^1-monoalkyldicyandiamides in which the same intermediates were brought into reaction with ammonia. The reaction was first explored with *N*-cyano-*S*-methyl-*N*′-*iso*propyl*iso*thiourea (VI; R = Pr^β) and *iso*propylamine. When these were heated together in alcoholic solution at 120° for 6 hours the product was shown to be N^1 : N^3-*di*iso*propyldicyandiamide* (VII; R = R′ = Pr^β) since it reacted with *p*-chloroaniline hydrochloride in boiling aqueous 2-ethoxyethanol solution to give N^1-p-*chlorophenyl*-N^4 : N^5-*di*iso*propyldiguanide* (III; R = R′ = Pr^β) *hydrochloride* identical with the product made by an alternative method described below.

Because of the equivalence of N^1 and N^3 in the dicyandiamide molecule it is obviously possible to proceed in two ways when utilising this method for the preparation of N^1 : N^3-dialkyldicyandiamides in which the alkyl groups are different. This is illustrated by the preparation of N^1-*ethyl*-N^3-iso*propyldicyandiamide*, not only by the action of ethylamine on *N*-cyano-*S*-methyl-*N*′-*iso*propyl*iso*thiourea (VI; R = Pr^β), but also from *iso*propylamine and *N*-cyano-*S*-methyl-*N*′-ethyl*iso*thiourea (VI; R = Et).

Parallel with the work described in Part XXIX (*loc. cit.*) on the condensation of aryl *iso*thiocyanates with mono- and di-alkylguanidines, we explored the reaction of alkyl *iso*thiocyanates with *p*-chlorophenylguanidine. *iso*Propyl *iso*thiocyanate, which was first investigated, reacted when merely heated with *p*-chlorophenylguanidine on the steam-bath. Analysis of the product indicated that it was the desired N-p-*chlorophenylguanyl*-N′-iso*propylthiourea* (VIII; R = Cl, R′ = Pr^β), but additional proof was provided by its conversion with alcoholic ammonia and lead monoxide at room temperature into N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (III; R = Pr^β, R′ = H) (Part X, *J.*, 1946, 729). The foregoing *N*-*p*-chlorophenylguanyl-*N*′-*iso*propylthiourea reacted analogously with *iso*propylamine, instead of ammonia, and a desulphurising agent to give N^1-*p*-chlorophenyl-N^4 : N^5-di*iso*propyldiguanide (III; R = R′ = Pr^β). Similarly, methyl *iso*thiocyanate reacted with *p*-chlorophenylguanidine to give (VIII; R = Cl, R′ = Me) from which N^1-*p*-chlorophenyl-N^4 : N^5-dimethyldiguanide (III; R = R′ = Me) (isolated as its *hydrochloride*) was obtained by the action of methylamine and mercuric oxide, and the identity of the product with that obtained by condensation of N^1 : N^3-*dimethyldicyandiamide* (VII; R = R′ = Me) with *p*-chloroaniline established.

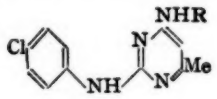
For the preparation of N^1-*p*-chlorophenyl-N^4-methyl-N^5-*iso*propyldiguanide, N-p-*chlorophenylguanyl*-N′-*methylthiourea* was condensed with *iso*propylamine in presence of mercuric oxide, although it was theoretically possible to proceed from *N*-*p*-chlorophenylguanyl-*N*′-*iso*propylthiourea and methylamine. This is due to the equivalence of N^4 and N^5 in the diguanide molecule and the fact that the alkyl groups involved are different.

By the above methods a series of N^1-*p*-chlorophenyl-N^4 : N^5-dialkyldiguanides were prepared for antimalarial test, the results of which will be published elsewhere. The dystherapeutic effect of a N^5-alkyl group was such that it did not appear worth while to prepare any N^1-aryl-N^4-alkyl-N^5-dialkyldiguanides, and the condensation of guanylthioureas of type (VIII) with dialkylamines was not therefore explored.

The *N*-*p*-chlorophenylguanyl-*N*′-alkylthioureas of type (VIII; R = Cl, R′ = alkyl) were tested for antimalarial activity by Dr. D. G. Davey using *P. gallinaceum* in chicks; all were inactive.

The conversion of *N*-*p*-chlorophenylguanyl-*N*′-*iso*propylthiourea (VIII; R = Cl, R′ = Pr^β) into N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide, referred to above, was also attempted *via* the corresponding *S*-methyl derivative (IX; R = Cl, R′ = Pr^β, R″ = SMe). This compound was obtained by the action of methyl iodide in methanol solution on (VIII; R = Cl, R′ = Pr^β) and also by condensation of *N*-cyano-*S*-methyl-*N*′-*iso*propyl*iso*thiourea with *p*-chloroaniline hydrochloride, although in poor yield. The latter type of reaction is reminiscent of the work

of Slotta, Tschesche, and Dressler (*Ber.*, 1930, **63**, 208), who condensed the sodium salt of *N*-cyano-*N'*-phenylthiourea with aniline hydrochloride in alcohol solution to obtain *N*-phenyl-*N'*phenylguanylthiourea.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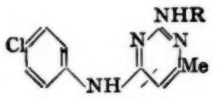
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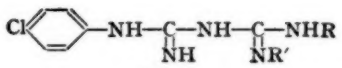
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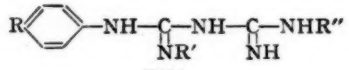
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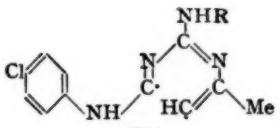
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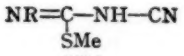
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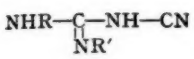
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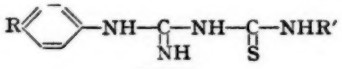
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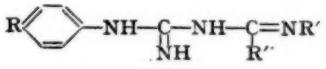
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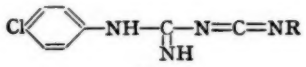
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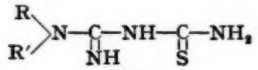
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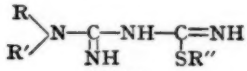
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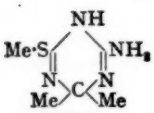
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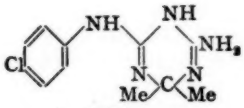
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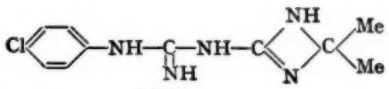
(XV.)

Although Slotta and Tschesche (*Ber.*, 1929, **62**, 1390) had described the reaction of *N*-guanyl-*N'*-methyl-*S*-ethyl*iso*thiourea hydrobromide with methylamine and dimethylamine respectively to give $N^1 : N^3$-dimethyldiguanide and $N^1 : N^1 : N^3$-trimethyldiguanide, when (IX; R = Cl, R' = Pr$^\beta$, R'' = SMe) was heated with saturated alcoholic ammonia for 6 hours at 100° no formation of N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide could be detected and analysis of the product (and of its *hydrochloride* and *acetate*) proved it to be N-p-*chlorophenylguanyl*-O-*ethyl*-N'-iso*propyl*iso*urea* (IX; R = Cl, R' = Pr$^\beta$, R'' = OEt). On repeating the reaction at a slightly higher temperature (120°) the same production of (IX; R = Cl, R' = Pr$^\beta$, R'' = OEt) was observed in one experiment, but in another some diguanide (III; R = Pr$^\beta$, R' = H) formation was demonstrated. The formation of *N*-*p*-chlorophenylguanyl-*O*-ethyl-*N'*-*iso*propyl*iso*urea when (IX; R = Cl, R' = Pr$^\beta$, R'' = SMe) is heated with alcoholic ammonia is most easily accounted for by the initial loss of methylthiol from the *N*-*p*-chlorophenylguanyl-*S*-methyl-*N'*-*iso*propyl*iso*thiourea followed by the addition of solvent, instead of ammonia, to the resulting carbodi-imide (X; R = Pr$^\beta$), and, in conformity with this (IX; R = Cl, R' = Pr$^\beta$, R'' = SMe), reacted normally with *iso*propylamine in the absence of a solvent to give N^1-*p*-chlorophenyl-$N^4 : N^5$-di*iso*propyldiguanide (III; R = R' = Pr$^\beta$).

These results suggest that the conversion of guanylthioureas of type (VIII; R = Cl, R' = alkyl) into diguanides of type (III; R = alkyl, R' = H or alkyl) by the action of ammonia or an alkylamine in presence of a desulphurising agent also proceeds *via* a carbodi-imide (X; R = alkyl), and other investigations, described below, which we have carried out on the conversion of guanylthioureas into diguanides tend to support this view. [By the action of mercuric oxide on *N*-phenyl-*N'*-(*NN'*-diphenylguanyl)thiourea Rathke and Oppenheim *Ber.*, 1890, **23**, 1669) isolated a substance which was considered to be a carbodi-imide.]

By analogy with the conversion (see above) of *N*-*p*-chlorophenylguanyl-*N'*-*iso*propylthiourea (VIII; R = Cl, R' = Pr$^\beta$) into the diguanide (III; R = R' = Pr$^\beta$) by reaction with methanolic *iso*propylamine and mercuric oxide at room temperature, it was thought that (VIII; R = Cl, R' = H) treated similarly might yield N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (IV; R = Cl, R' = H, R'' = Pr$^\beta$). Actually it was found that the main reaction was the conversion of p-*chlorophenylguanylthiourea* into *p*-chlorophenyldicyandiamide. Some unchanged (VIII; R = Cl, R' = H) was also isolated, and in addition a small more highly basic fraction was separated in which it was possible to detect some $N^1 : N^5$-disubstituted diguanide by shaking

with ammoniacal copper sulphate and benzene, when a pink colour developed in the benzene owing to formation of a benzene-soluble copper complex of the diguanide (cf., Gage and Rose, *Ann. Trop. Med. Parasit.*, 1946, **40**, 333). Following this, isolation of a very small amount of N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (IV; R = Cl, R′ = H, R″ = Pr$^\beta$) as its acetate was achieved. Many similar experiments (not detailed) were performed, with ammonia, methylamine, dimethylamine, or methyl*iso*propylamine in place of *iso*propylamine, and with different desulphurising agents such as mercuric chloride and copper sulphate; the temperature was also varied from 10° to 65°. In every case *p*-chlorophenyldicyandiamide was the main product, and the yield of diguanide, though variable, was never more than 5%, and often considerably less. The formation of these small amounts of diguanides by direct interaction of (VIII; R = Cl, R′ = H) with the alkylamines, although not definitely excluded, appeared unlikely since it was separately demonstrated that a small yield of diguanide resulted from the interaction of *p*-chlorophenyldicyandiamide with methanolic *iso*propylamine under similar conditions, either with or without mercuric oxide. Furthermore, it was subsequently shown that higher yields of diguanides were obtained from arylguanylthioureas by reaction with alkylamine hydrochlorides and a desulphurising agent under conditions previously found suitable (Part XXVIII, *loc. cit.*) for the reaction of aryldicyandiamides with alkylamines. Thus *p*-chlorophenylguanylthiourea and *iso*propylamine hydrochloride reacted in nitrobenzene at 130—135° in presence of mercuric oxide to give N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide, and *p*-iodophenylguanylthiourea (VIII; R = I, R′ = H) was similarly converted into N^1-*p*-iodophenyl-N^5-*iso*propyldiguanide (IV; R = I, R′ = H, R″ = Pr$^\beta$).

The desulphurisation of *p*-chlorophenylguanylthiourea to give *p*-chlorophenyldicyandiamide was also effected by means of mercuric oxide in boiling methanol solution without any *iso*propylamine, although the reaction was less facile than in the presence of the amine. This conversion is undoubtedly analogous to that of *N*-(*N*-phenyl-*N′*-*p*-tolylguanyl)thiourea into N^1-phenyl-N^2-*p*-tolyldicyandiamide (Fromm and Weller, *Annalen*, 1908, **361**, 306) and of *N*-(*NN′*-di-*p*-methoxyphenylguanyl)thiourea into N^1 : N^2-di-*p*-methoxyphenyldicyandiamide (Fromm, *ibid.*, 1912, **394**, 258) by the action of lead monoxide under alkaline conditions. Even earlier, Rathke (*Ber.*, 1878, **11**, 962) had demonstrated the conversion of guanylthiourea itself into dicyandiamide by the action of silver nitrate.

Our failure to obtain N^1-*p*-methoxyphenyl-N^5-*iso*propyldiguanide by interaction of iso*propylguanylthiourea* (XI; R = Pr$^\beta$, R′ = H) with *p*-anisidine in methanol solution at 50—60° in presence of mercuric oxide can probably likewise be attributed to the ready desulphurisation of the guanylthiourea to give *iso*propyldicyandiamide (VII; R = Pr$^\beta$, R′ = H) and the unsuitability of the conditions to effect condensation of the dicyandiamide with the *p*-anisidine. In support of this it was demonstrated that treatment of (XI; R = Pr$^\beta$, R′ = H) with mercuric oxide in alcoholic solution at 55—60° in presence of trimethylamine gave rise to *iso*propyldicyandiamide together with a second substance having the empirical formula $C_5H_{10}N_4S$ which was assumed to be one, or possibly a mixture, of the various possible oxidation products of (XI; R = Pr$^\beta$, R′ = H) (a thiadiazole or dihydro-1 : 2 : 4-triazole-thione). Successful conditions which were later discovered for the condensation of *iso*propylguanylthiourea and *p*-bromoaniline hydrochloride in presence of mercuric oxide to give N^1-*p*-bromophenyl-N^5-*iso*propyldiguanide (IV; R = Br, R′ = H, R″ = Pr$^\beta$) resembled those employed for the condensation of alkyldicyandiamides with arylamines (Part XXVIII).

For comparison, and in view of the work of Slotta and Tschesche (*Ber.*, 1929, **62**, 1398), who prepared a number of substituted alkyldiguanides and alkylene bisdiguanides by the reaction of guanyl-*S*-ethyl*iso*thiourea hydrobromide with alkylamines and alkylenediamines, it was decided to explore the reaction of arylguanyl-*S*-alkyl*iso*thioureas of type (IX; R′ = H, R″ = S·Alkyl) with alkylamines and of alkylguanyl-*S*-alkyl*iso*thioureas of type (XII; R = R″ = alkyl, R′ = H or alkyl) with arylamines. Accordingly the condensation of p-*chlorophenylguanyl*-S-*methylisothiourea hydriodide* (as IX; R = Cl, R′ = H, R″ = SMe) with various alkylamines was investigated. Using methanol as the solvent and carrying out the reaction in the cold with a reaction time of 4—7 days, the yields of diguanide were very variable—20% with *iso*propylamine and 90% with dimethylamine—and this is probably attributable to the facility of the desired reaction (elimination of methylthiol between the two reactants) compared with that of *p*-chlorophenyldicyandiamide formation. This substance was the main product of many of the reactions tried, and its formation appeared to be favoured by the presence of material quantities of water (illustrated by the reactions using methylamine).

The effect of water was again observed in a number of reactions carried out at higher temperatures with a view to promoting condensation of the amine with the *p*-chlorophenyldi-

cyandiamide formed. *N*-*p*-Chlorophenylguanyl-*S*-methyl*iso*thiourea hydriodide and *iso*propylamine under anhydrous conditions in nitrobenzene at 130—135° gave little or no diguanide, whereas at the same temperature in presence of water they gave a 70% yield of N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide.

Attention was then directed to the condensation of salts of *N*-alkylguanyl-*S*-alkyl*iso*thioureas (XII; R = R″ = alkyl, R′ = H or alkyl) with arylamines, and it was found that such reactions were successfully effected under conditions favouring the condensation of alkyldicyandiamides with arylamines. This can, however, only be regarded as presumptive evidence, and not positive proof, that the reactions proceeded *via* the alkyldicyandiamides formed by loss of alkylthiol from the *N*-alkylguanyl-*S*-alkyl*iso*thioureas. The reactions of this type included condensation of the *hydriodide* of N-iso*propylguanyl*-S-*methyl*iso*thiourea* (XII; R = Pr^{β}, R′ = H R″ = Me) with *p*-chloroaniline in boiling aqueous solution to give N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide, of N-(N-*methyl*-N-iso*propylguanyl*)-S-*methyl*iso*thiourea* (XII; R = Pr^{β}, R′ = R″ = Me) *hydriodide* with *p*-chloroaniline to give N^1-*p*-chlorophenyl-N^5-methyl-N^5-*iso*propyldiguanide, and of *N*-*n*-butylguanyl-*S*-methyl*iso*thiourea (XII; R = Bu^{α}, R′ = H, R″ = Me) as its hydriodide with *p*-bromoaniline to give N^1-*p*-bromophenyl-N^5-*n*-butyldiguanide (IV; R = Br, R′ = H, R″ = Bu^{α}).

The above investigations utilising aryl- and alkyl-guanylthioureas were preceded by a considerable amount of work on the synthesis of such compounds. Under conditions similar to those used for the preparation of diguanides from cyanamides and guanidines (forthcoming publication), dimethylcyanamide could not be induced to react with thiourea to give *NN*-dimethylguanylthiourea. Instead, dimethylthiourea and dimethylguanidine (identified as it spicrate) were formed, presumably by the addition of the thermal decomposition products of thiourea to the dimethylcyanamide.

The method of preparation of guanylthiourea described in U.S.P. 2,364,594, which involves the reaction of dicyandiamide with carbon disulphide and potassium hydroxide to give dipotassium ω-cyanoguanidinodithiocarbonate followed by ring closure of this with acetic acid and hydrolysis of the resulting 4 : 6-diamino-2-mercapto-1 : 3 : 5-thiadiazine with a strong acid, was confirmed, but the application of this method to aryl- and alkyl-dicyandiamides failed at the first stage. Both *p*-chlorophenyldicyandiamide and *iso*propyldicyandiamide afforded only tars on treatment with carbon disulphide and potassium hydroxide, but the method was not exhaustively investigated because it was found that the addition of hydrogen sulphide to a dicyandiamide provided a very convenient method for the preparation of the required aryl- and alkyl-guanylthioureas.

Bamberger (*Ber.*, 1883, **16**, 1460) and Slotta and Tschesche (*ibid.*, 1929, **62**, 1398) used this route for the preparation of the parent guanylthiourea. Their method was modified slightly, and the dicyandiamides were heated with a methanol solution of hydrogen sulphide (at least two equivalents) at 70—80° for at least 48 hours. Initially a catalytic amount of sodium was added (cf. Kinder, *Annalen*, 1923, **431**, 187; the addition of hydrogen sulphide to nitriles), but in later preparations this was omitted with no lowering of the yield. By this method *p*-chlorophenylguanylthiourea and *p*-iodophenylguanylthiourea were made, besides the monoalkyl compounds, *iso*propyl (XI; R = Pr^{β}, R′ = H) and n-*butylguanylthiourea* (XI; R = Bu^{α}, R′ = H), and the dialkyl derivatives NN-*diethyl*- (XI; R = R′ = Et) and N-*methyl*-N-iso*propyl-guanylthiourea* (XI; R = Me, R′ = Pr^{β}). In the case of *p*-chlorophenylguanylthiourea a small amount of p-*chlorophenyldithiobiuret* was isolated, identical with that made by condensing *p*-chloroaniline with "xynthane hydride" (perthiocyanic acid) (cf. Glutz, *Annalen*, 1870, **154**, 44; Tursini, *Ber.*, 1884, **17**, 584; Fromm *et al.*, *Annalen*, 1893, **275**, 20; 1906, **348**, 161; *Ber.*, 1895, **28**, 1096; Underwood and Dains, *J. Amer. Chem. Soc.*, 1935, **57**, 1768), and by interaction of *p*-chlorophenyl *iso*thiocyanate with *S*-methyl*iso*thiourea followed by thiohydrolysis of the product (method of Johnson *et al.*, *Amer. Chem. J.*, 1903, **30**, 167; Olin and Dains, *J. Amer. Chem. Soc.*, 1930, **52**, 3326; Underwood and Dains, *Kansas Univ. Sci. Bull.*, 1936, **24**, 5). It may be significant that the sodium catalyst was used in this preparation, although U.S.P. 2,371,112 describes the preparation of dithiobiuret itself by heating dicyandiamide with hydrogen sulphide under pressure in presence of an inert solvent.

Besides *p*-chlorophenylguanyl-*S*-methyl*iso*thiourea, mentioned above, a number of homologous compounds (IX; R = Cl, R′ = H, R″ = SEt, SPr^{β}, $S{\cdot}CH_2Ph$) were prepared in order to permit their examination for antimalarial activity. This was thought to be of interest on account of their formal analogy to the diguanides of type (III; R = alkyl, R′ = H) and because they likewise afford benzene-soluble copper complexes. However, none of the compounds exhibited any activity against *P. gallinaceum* in chicks.

The guanylthioureas, both alkyl and aryl, were *S*-alkylated by treatment with alkyl halides. The alkylguanylthioureas (XI; R = alkyl, R′ = H or alkyl) reacted with methyl iodide in the cold, and the arylguanylthioureas (VIII; R′ = H) after 2—6 hours at 60—65°. The reaction between *p*-chlorophenylguanylthiourea and *iso*propyl bromide to give (IX; R = Cl, R′ = H, R″ = SPr$^{\beta}$) required 24 hours at 70—80° for completion. An attempt was also made to prepare this type of compound by the reaction of cyanamides with *S*-alkyl*iso*thioureas. It was thought that this might be more successful than with the parent thiourea, since the *S*-alkyl compounds are stronger bases, and in this respect resemble guanidines. Owing to the instability of the *S*-alkyl*iso*thioureas, however, the use of very mild conditions was indicated, and the condensation of dimethylcyanamide and of methyl*iso*propylcyanamide with *S*-methyl*iso*thiourea was attempted in acetone. No guanyl-*S*-alkyl*iso*thioureas were obtained: instead, both reactions gave a 20—30% yield of a substance which gave the correct analysis for *2-amino-4-methylthio-6-dimethyldihydro-1 : 3 : 5-triazine* (XIII) which could have been formed by reaction of 2 mols. of *S*-methyl*iso*thiourea with the acetone used as solvent. This was later substantiated experimentally. The supposed constitution of (XIII) was further supported by the fact that it reacted with *p*-chloroaniline to give a product identical with that obtained by the reaction of *p*-chlorophenyldiguanide and acetone which, from its properties, would appear to be *2-amino-4-p-chloroanilino-6-dimethyldihydro-1 : 3 : 5-triazine* (XIV). It does not form a copper complex, and this is regarded as evidence for the condensation of the acetone across N^{2} and N^{4}, since *N*^{1}-aryl-*N*^{2} : *N*^{5}-dialkyl- and *N*^{1}-aryl-*N*^{4} : *N*^{5}-dialkyl-diguanides retain this property as does 2-*p*-chlorophenylguanidino-4-β-diethylaminoethylamino-6-methylpyrimidine (Part IV, *J.*, 1946, 362), and this fact appears to exclude the alternative structure (XV). The simple elimination of water between the acetone and one of the terminal amino-groups appears to be excluded not only by the non-formation of a copper-complex but also by the inactivity of the substance as an antimalarial, and our inability to effect its reduction to *N*^{1}-*p*-chlorophenyl-*N*^{5}-*iso*propyldiguanide.

Experimental.

N^{1} : N^{2}-*Diisopropyldicyandiamide* (VII; R = R′ = Pr$^{\beta}$).—*N*-Cyano-*S*-methyl-*N′-iso*propyl*iso*thiourea (4 g.) (Part XXVIII, *loc. cit.*), *iso*propylamine (15 c.c.), and alcohol (45 c.c.) were heated in a sealed tube at 120° for 6 hours. The contents of the tube were then evaporated to half their original volume and set aside to crystallise. The material which separated was collected and recrystallised from alcohol giving the *product* as colourless leaflets, m. p. 194° (Found: C, 57·2; H, 9·4; N, 32·6. $C_8H_{16}N_4$ requires C, 57·15; H, 9·5; N, 33·3%).

N^{1}-*Ethyl*-N^{2}-iso*propyldicyandiamide* (VII; R = Et, R′ = Pr$^{\beta}$).—(*a*) *N*-Cyano-*S*-methyl-*N′-iso*propyl*iso*thiourea (4 g.), ethylamine (20 c.c. of 33%), and alcohol (40 c.c.) were heated at 120° for 6 hours in a closed vessel. The mixture was then evaporated to dryness and the residue crystallised from light petroleum (b. p. 80—100°)–*n*-propanol to give N^{1}-*ethyl*-N^{2}-iso*propyldicyandiamide* as colourless leaflets, m. p. 132—133° (Found: C, 54·6; H, 9·3; N, 36·0. $C_7H_{14}N_4$ requires C, 54·55; H, 9·1; N, 36·4%).

(*b*) *N*-Cyano-*S*-methyl-*N′*-ethyl*iso*thiourea (4 g.), *iso*propylamine (15 c.c.), and alcohol (25 c.c.) were heated for 6 hours at 120° and worked up as described under (*a*) to give the same compound, m. p. and mixed m. p. 133°.

N^{1} : N^{2}-*Dimethyldicyandiamide* (VII; R = R′ = Me).—Prepared similarly from *N*-cyano-*N′S*-dimethyl*iso*thiourea and methylamine, this *compound* crystallised from *n*-propanol as colourless leaflets, m. p. 174—175° (Found: C, 42·9; H, 7·0; N, 49·6. $C_4H_8N_4$ requires C, 42·9; H, 7·1; N, 50·0%).

N^{1}-*Methyl*-N^{2}-*ethyldicyandiamide* (VII; R = Me, R′ = Et).—Prepared from *N*-cyano-*N′S*-dimethyl*iso*thiourea and ethylamine, this *compound* crystallised from water as colourless prisms, m. p. 98—99° (Found: C, 47·5; H, 7·9; N, 44·7. $C_5H_{10}N_4$ requires C, 47·6; H, 7·9; N, 44·4%).

N^{1}-*Methyl*-N^{2}-n-*butyldicyandiamide* (VII; R = Me, R′ = Bu$^{\alpha}$).—Prepared from *N*-cyano-*N′S*-dimethyl*iso*thiourea and *n*-butylamine, this *compound* formed small colourless prisms, m. p. 91—92° (Found: C, 54·6; H, 9·0; N, 36·5. $C_7H_{14}N_4$ requires C, 54·55; H, 9·1; N, 36·4%).

N^{1}-n-*Propyl*-N^{2}-iso*propyldicyandiamide* (VII; R = Pr$^{\alpha}$, R′ = Pr$^{\beta}$).—Prepared from *N*-cyano-*S*-methyl-*N′-iso*propyl*iso*thiourea and *n*-propylamine, this *compound* crystallised from light petroleum (b. p. 80—100°)–*iso*propanol as colourless prisms, m. p. 131° (Found: C, 57·1; H, 9·7; N, 32·9. $C_8H_{16}N_4$ requires C, 57·15; H, 9·5; N, 33·3%).

N^{1}-iso*Propyl*-N^{2}-n-*butyldicyandiamide* (VII; R = Pr$^{\beta}$, R′ = Bu$^{\alpha}$).—Prepared from *N*-cyano-*S*-methyl-*N′-iso*propyl*iso*thiourea and *n*-butylamine, this *compound* separated from *iso*propanol–light petroleum as colourless prisms, m. p. 104—105° (Found: C, 59·7; H, 9·8; N, 30·5. $C_9H_{18}N_4$ requires C, 59·3; H, 9·9; N, 30·8%).

N^{1}-iso*Propyl*-N^{2}-cyclo*hexyldicyandiamide* (VII; R = Pr$^{\beta}$, R′ = C_6H_{11} *cyclo*).—Prepared from *N*-cyano-*S*-methyl-*N′-iso*propyl*iso*thiourea and *cyclo*hexylamine, this *compound* formed colourless prisms from alcohol, m. p. 192° (Found: C, 63·3; H, 9·4; N, 26·6. $C_{11}H_{20}N_4$ requires C, 63·5; H, 9·6; N, 26·9%).

N-p-*Chlorophenylguanyl*-N′-iso*propylthiourea* (VIII; R = Cl, R′ = Pr$^{\beta}$).—*p*-Chlorophenylguanidine (17 g.) (for convenient method of preparation see Part XXV, this vol., p. 586) and *iso*propyl *iso*thiocyanate (12 g.) were heated on the steam-bath for 1½ hours, a homogeneous mixture being quickly obtained. The excess of *iso*propyl *iso*thiocyanate was removed by steam distillation, and 2N-hydrochloric acid (55 c.c.) added to the hot residue. The resulting solid was filtered off when cold, dried, and extracted

thoroughly with ethyl acetate in the cold. The insoluble material crystallised from dilute hydrochloric acid to give N-p-*chlorophenylguanyl*-N′-iso*propylthiourea hydrochloride* (yield, 20·6 g.) as colourless prisms, m. p. 178° (Found: C, 43·0; H, 5·0; N, 18·2; Cl, 22·6; S, 10·7. $C_{11}H_{15}N_4ClS$,HCl requires C, 43·0; H, 5·2; N, 18·2; Cl, 23·1; S, 10·4%) (5168). The *base* was obtained by treating a solution of the hydrochloride in hot water with sodium hydroxide solution, boiling for a few minutes, and cooling. After drying, it crystallised from benzene–light petroleum (b. p. 80—100°) as colourless prisms, m. p. 100—101° (Found: C, 49·1; H, 5·2; N, 20·8. $C_{11}H_{15}N_4ClS$ requires C, 48·8; H, 5·5; N, 20·7%).

Conversion of N-p-*Chlorophenylguanyl*-N′-iso*propylthiourea into* N¹-p-*Chlorophenyl*-N⁵-iso*propyldiguanide*.—The above hydrochloride (2 g.), lead monoxide (4 g.), and saturated alcoholic ammonia (20 c.c.) were stirred vigorously overnight. The mixture was then filtered and the residue washed with alcohol. The solvent was removed from the filtrate by evaporation, and the residue dissolved in acetone. After filtration to remove a little insoluble material, acetic acid was added so as just to destroy the alkalinity to brilliant-yellow. After ½ hour the resulting precipitate was filtered off, washed with acetone, and dried to give *N*¹-*p*-chlorophenyl-*N*⁵-*iso*propyldiguanide acetate (yield, 0·5 g.), m. p. 185°, undepressed in admixture with authentic material (see Part X, *J.*, 1946, 729) (Found: C, 49·5; H, 5·9; N, 22·3. Calc. for $C_{11}H_{16}N_5Cl,CH_3{\cdot}CO_2H$: C, 49·7; H, 6·4; N, 22·3%).

N-p-*Chlorophenylguanyl*-N′-*methylthiourea* (VIII; R = Cl, R′ = Me).—Similarly prepared from methyl *iso*thiocyanate and *p*-chlorophenylguanidine, the *base* crystallised from alcohol in small colourless needles, m. p. 118° (Found: C, 44·4; H, 4·5; N, 23·2. $C_9H_{11}N_4ClS$ requires C, 44·5; H, 4·5; N, 23·1%), and the *hydrochloride* (5308) separated from dilute hydrochloric acid as colourless needles, m. p. 177—178° (Found: C, 37·8; H, 4·5; N, 19·3; Cl, 24·7; S, 11·4. $C_9H_{11}N_4ClS$,HCl,0·5H_2O requires C, 37·5; H, 4·5; N, 19·4; Cl, 24·6; S, 11·1%).

N-p-*Chlorophenylguanyl*-N′-*ethylthiourea* (VIII; R = Cl, R′ = Et).—The *hydrochloride* (5381), prepared from ethyl *iso*thiocyanate and *p*-chlorophenylguanidine, crystallised from water as long colourless prisms, m. p. 177° (Found: C, 41·1; H, 4·6; N, 19·2. $C_{10}H_{13}N_4ClS$,HCl requires C, 41·0; H, 4·8; N, 19·1%). The *base* crystallised from *iso*propanol in small colourless prisms, m. p. 97—98° (Found: C, 46·8; H, 5·2; N, 21·7. $C_{10}H_{13}N_4ClS$ requires C, 46·8; H, 5·1; N, 21·8%).

N-p-*Chlorophenylguanyl*-N′-n-*propylthiourea* (VIII; R = Cl, R′ = Pr[α]).—The *hydrochloride*, prepared from *p*-chlorophenylguanidine and *n*-propyl *iso*thiocyanate, crystallised from dilute hydrochloric acid and then from acetone as long colourless needles, m. p. 171° (Found: C, 43·1; H, 5·2; N, 17·7; S, 10·0. $C_{11}H_{15}N_4ClS$,HCl requires C, 43·0; H, 5·2; N, 18·2; S, 10·4%) (5288). The *base*, liberated from a hot aqueous solution of the hydrochloride with sodium hydroxide, crystallised from alcohol as colourless prisms, m. p. 116° (Found: C, 48·9; H, 5·5; N, 20·4. $C_{11}H_{15}N_4ClS$ requires C, 48·8; H, 5·5; N, 20·7%).

N-p-*Chlorophenylguanyl*-N′-n-*butylthiourea* (VIII; R = Cl, R′ = Bu[α]).—The *hydrochloride*, prepared from *n*-butyl *iso*thiocyanate and *p*-chlorophenylguanidine, crystallised from dilute hydrochloric acid as colourless prisms, m. p. 172° (Found: C, 45·5; H, 6·0; N, 17·5; S, 10·1. $C_{12}H_{17}N_4ClS$,HCl requires C, 44·9; H, 5·6; N, 17·45; S, 10·0%) (5289). A solution of the hydrochloride in hot water was made alkaline with ammonia and the liberated base extracted with benzene. Evaporation of the dried (K_2CO_3) benzene solution left an oil which solidified on trituration with light petroleum (b. p. 80—100°). Crystallisation of this solid from *iso*propanol gave N-p-*chlorophenylguanyl* N′-n-*butylthiourea* as colourless prisms, m. p. 97—98° (Found: C, 50·6; H, 6·0; N, 19·6. $C_{12}H_{17}N_4ClS$ requires C, 50·6; H, 6·0; N, 19·7%).

N¹-p-*Chlorophenyl*-N⁴ : N⁵-*di*iso*propyldiguanide* (III; R = R′ = Pr[β]).—(*a*) *N*-*p*-Chlorophenylguanyl-*N*′-*iso*propylthiourea (5·4 g.), *iso*propylamine (5 g.), methanol (15 c.c.), and mercuric oxide (8·6 g.) were stirred at room temperature for 16 hours. The mixture was then filtered and the inorganic residue washed well with methanol. The filtrate and washings were evaporated to dryness. The solid residue was dissolved in ethyl acetate (50 c.c.), and the alkalinity of the solution to brilliant-yellow removed by addition of alcoholic hydrogen chloride. The precipitated *hydrochloride* was collected, dried, and crystallised from alcohol; it formed colourless prisms, m. p. 254° (Found: C, 50·9; H, 7·1; N, 21·2; Cl, 20·9; Cl′, 10·4. $C_{14}H_{23}N_5Cl$,HCl requires C, 50·6; H, 6·9; N, 21·1; Cl, 21·4; Cl′, 10·7%) (5301). The *base*, obtained by the addition of sodium hydroxide to a hot aqueous solution of the hydrochloride, crystallised from light petroleum (b. p. 100—120°) as colourless prisms, m. p. 136° (Found: C, 57·1; H, 7·3; N, 23·6; Cl, 12·3. $C_{14}H_{23}N_5Cl$ requires C, 56·9; H, 7·4; N, 23·7; Cl, 12·0%).

(*b*) *N*¹ : *N*⁵-Di*iso*propyldicyandiamide (0·2 g.), *p*-chloroaniline hydrochloride (0·3 g.), water (5 c.c.), and 2-ethoxyethanol (1 c.c.) were heated at the boil for 3 hours. The product which separated on cooling was filtered off and dissolved in excess of hydrochloric acid. The filtered solution was treated with ammonia to reduce the acidity until it was just acid to Congo-red. On standing, the hydrochloride separated and was purified by crystallisation from water to give the same product as in (*a*), m. p. and mixed m. p. 254°.

N¹-p-*Chlorophenyl*-N⁴ : N⁵-*dimethyldiguanide* (III; R = R′ = Me).—(*a*) A mixture of *N*-*p*-chlorophenylguanyl-*N*′-methylthiourea (5 g.), methylamine hydrochloride (6·75 g.), sodium methoxide (5·4 g.), methanol (30 c.c.), and mercuric oxide (8·6 g.) was stirred for 18 hours. It was then filtered and the solution evaporated to dryness. The residue was dissolved in a mixture of equal parts of water and hydrochloric acid and the solution treated with sodium hydroxide until it remained only faintly acid to Congo-red. The precipitated *hydrochloride* (3 g.) crystallised from water as colourless prisms, m. p. 249—250° (Found: C, 43·8; H, 5·2; N, 25·2. $C_{10}H_{14}N_5Cl$,HCl requires C, 43·5; H, 5·4; N, 25·4%) (6093).

(*b*) *N*¹ : *N*⁵-Dimethyldicyandiamide (2 g.), *p*-chloroaniline hydrochloride (3 g.), and water (20 c.c.) were refluxed for 1½ hours. The product which separated on cooling was collected, washed with a little 2-ethoxyethanol, and crystallised from water to give *N*¹-*p*-chlorophenyl-*N*⁴ : *N*⁵-dimethyldiguanide hydrochloride, m. p. and mixed m. p. 250° (Found: C, 43·8; H, 5·4; N, 25·3%).

N¹-p-*Chlorophenyl*-N⁴-*methyl*-N⁵-*ethyldiguanide* (III; R = Me, R′ = Et).—*N*¹-Methyl-*N*⁵-ethyldicyandiamide (3 g.) and *p*-chloroaniline hydrochloride (4 g.) were boiled with water (20 c.c.) for 1½ hours. After cooling, the product which had separated was filtered off, dried, and triturated with ethyl acetate.

It then crystallised from water to give the *hydrochloride* (6094) as colourless prisms, m. p. 215° (Found: C, 45·6; H, 5·8; N, 24·2. $C_{11}H_{16}N_5Cl,HCl$ requires C, 45·5; H, 5·9; N, 24·1%).

N¹-p-*Chlorophenyl*-N⁴-*methyl*-N⁵-*isopropyldiguanide* (III; R = Me, R′ = Prβ).—*N*-*p*-Chlorophenylguanyl-*N*′-methylthiourea hydrochloride (5·6 g.), methanol (15 c.c.), and sodium methoxide (1·08 g.) were stirred for 10 minutes. *iso*Propylamine (5 g.) and mercuric oxide (8·6 g.) were then added and the stirring continued for 18 hours. The mixture was then filtered and the inorganic residue washed with methanol. The filtrate and washings were evaporated to dryness and the residue dissolved in 2N-hydrochloric acid. The resulting solution, after filtration, was treated with sodium hydroxide so as just to destroy the Congo-red acidity. The precipitated *hydrochloride* crystallised from water as small colourless prisms, m. p. 205—206° (Found: C, 47·4; H, 6·3; N, 22·7. $C_{12}H_{18}N_5Cl,HCl$ requires C, 47·4; H, 6·25; N, 23·0%) (5328).

N¹-p-*Chlorophenyl*-N⁴-*methyl*-N⁵-n-*propyldiguanide* (III; R = Me, R′ = Prα).—*N*-*p*-Chlorophenylguanyl-*N*′-*n*-propylthiourea (5·4 g.), methylamine hydrochloride (6·75 g.), sodium methoxide (5·4 g.), methanol (15 c.c.), and mercuric oxide (8·6 g.) were stirred for 16 hours and the mixture then worked up as described above. The *hydrochloride* (5322) crystallised from water as colourless prisms, m. p. 164° (Found: C, 47·2; H, 5·9; N, 22·7. $C_{12}H_{18}N_5Cl,HCl$ requires C, 47·4; H, 6·25; N, 23·0%).

N¹-p-*Chlorophenyl*-N⁴-*methyl*-N⁵-n-*butyldiguanide* (III; R = Me, R′ = Buα).—(*a*) *N*¹-Methyl-*N*²-*n*-butyldicyandiamide (2 g.), *p*-chloroaniline hydrochloride (3·2 g.), and water (20 c.c.) were boiled under reflux for 1½ hours. The *hydrochloride* which separated on cooling was purified by crystallisation from water; it formed small colourless prisms, m. p. 175—176° (Found: C, 49·3; H, 6·6; N, 21·9. $C_{13}H_{20}N_5Cl,HCl$ requires C, 49·1; H, 6·6; N, 22·0%) (5321).

(*b*) Obtained from *N*-*p*-chlorophenylguanyl-*N*′-*n*-butylthiourea and methylamine as described above for the corresponding *n*-propyl compound, the hydrochloride had m. p. and mixed m. p. 175—176° (Found: C, 48·8; H, 6·4; N, 22·3%).

N¹-p-*Chlorophenyl*-N⁴-*ethyl*-N⁵-iso*propyldiguanide* (III; R = Et; R′ = Prβ).—Prepared from *N*¹-ethyl-*N*²-*iso*propyldicyandiamide and *p*-chloroaniline, the *hydrochloride* crystallised from water as colourless needles, m. p. 234—235° (Found: C, 49·0; H, 6·6; N, 21·8. $C_{13}H_{20}N_5Cl,HCl$ requires C, 49·1; H, 6·6; N, 22·0%) (5569).

N¹-p-*Chlorophenyl*-N⁴-n-*propyl*-N⁵-iso*propyldiguanide* (III; R = Prα, R′ = Prβ).—Prepared from *N*¹-*n*-propyl-*N*²-*iso*propyldicyandiamide and *p*-chloroaniline hydrochloride in boiling aqueous 2-ethoxyethanol solution, the *hydrochloride* formed colourless needles, m. p. 235—236° (Found: C, 50·9; H, 7·1; N, 21·3. $C_{14}H_{22}N_5Cl,HCl$ requires C, 50·6; H, 6·9; N, 21·1%).

N¹-p-*Chlorophenyl*-N⁵-cyclo*hexyl*-N⁴-iso*propyldiguanide* (III; R = Prβ, R′ = C_6H_{11} *cyclo*).—Prepared from *N*¹-*cyclo*hexyl-*N*²-*iso*propyldicyandiamide and *p*-chloroaniline hydrochloride, the *hydrochloride* crystallised from 2-ethoxyethanol as small colourless prisms, m. p. 238—239° (Found: C, 54·7; H, 7·3; N, 18·6. $C_{17}H_{26}N_5Cl,HCl$ requires C, 54·8; H, 7·3; N, 18·8%) (5592).

N¹-p-*Chlorophenyl*-N⁴-n-*propyl*-N⁵-n-*butyldiguanide* (III; R = Prα, R′ = Buα).—Prepared from *N*-*p*-chlorophenylguanyl-*N*′-*n*-propylthiourea and *n*-butylamine, the *hydrochloride* crystallised from water as small colourless needles, m. p. 163—164° (Found: C, 52·0; H, 7·0; N, 20·2. $C_{15}H_{24}N_5Cl,HCl$ requires C, 52·1; H, 7·2; N, 20·2%) (5386).

N¹-p-*Ethoxyphenyl*-N⁴-iso*propyl*-N⁵-n-*butyldiguanide*.—Prepared from *N*¹-*iso*propyl-*N*²-*n*-butyldicyandiamide and *p*-phenetidine hydrochloride, the *hydrochloride* crystallised from water as colourless prisms, m. p. 199° (Found: C, 57·6; H, 8·4; N, 19·9. $C_{17}H_{29}ON_5,HCl$ requires C, 57·4; H, 8·4; N, 19·7%) (5570).

N¹-(6-*Methoxy*-8-*quinolyl*)-N⁴-n-*propyl*-N⁵-iso*propyldiguanide*.—*N*¹-*n*-Propyl-*N*²-*iso*propyldicyandiamide (3 g.), 8-amino-6-methoxyquinoline hydrochloride (4·5 g.), and 2-ethoxyethanol (15 c.c.) were boiled under reflux for 3 hours. The *hydrochloride* which separated on cooling and adding ethyl acetate was purified by crystallisation from water; it formed pale yellow prisms, m. p. 254° (Found: C, 56·8; H, 7·1; N, 22·0. $C_{18}H_{26}ON_6,HCl$ requires C, 57·1; H, 7·1; N, 22·2%) (5752).

N-p-*Chlorophenylguanyl*-S-*methyl*-N′-iso*propyliso*thiourea (IX; R = Cl, R′ = Prβ, R″ = SMe).—(*a*) *N*-*p*-Chlorophenylguanyl-*N*′-*iso*propylthiourea (8·6 g.), methyl iodide (8 g.), and methanol (40 c.c.) were refluxed for 2 hours and the solution then evaporated to dryness under reduced pressure. The residue was warmed with water to *ca.* 50° and made alkaline to brilliant-yellow with ammonia. The resulting solid was collected, washed with water, and crystallised from dilute alcohol, giving colourless prisms (9·2 g.), m. p. 92° (Found: C, 50·9; H, 5·9. $C_{12}H_{17}N_4ClS$ requires C, 50·6; H, 6·0%). A solution of the *base* in warm dilute hydrochloric acid deposited the *hydrochloride* on cooling; recrystallised from dilute hydrochloric acid it formed colourless prisms, m. p. 201—202° (Found: C, 44·8; H, 5·5; N, 17·6. $C_{12}H_{17}N_4ClS,HCl$ requires C, 44·9; H, 5·6; N, 17·45%) (5593).

(*b*) *p*-Chloroaniline (1·27 g.) was dissolved in water (30 c.c.) by the addition of hydrochloric acid to give a solution just acid to Congo-red. This was added to *N*-cyano-*S*-methyl-*N*′-*iso*propyl*iso*thiourea (1·57 g.), and a solution of copper sulphate (1 g.) in the minimum quantity of water was added, followed by dioxan (10 c.c.). The mixture was refluxed for 1 hour, cooled, and filtered. The filtrate was extracted with chloroform and then treated with sodium chloride. The precipitated solid was collected and crystallised from water, giving the same hydrochloride (0·3 g.), m. p. 200° (Found: C, 44·5; H, 5·6; N, 17·8; S, 9·7. $C_{12}H_{17}N_4ClS,HCl$ requires S, 10·0%).

Reaction of N-p-*Chlorophenylguanyl*-S-*methyl*-N′-iso*propyliso*thiourea *with Alcoholic Ammonia*.—(*a*) The preceding compound (2 g.) and saturated alcoholic ammonia (50 c.c.) were heated for 6 hours at 120° in a sealed tube. The contents of the tube were evaporated to dryness and the residue stirred with 2N-hydrochloric acid. The resulting solid was filtered off. The filtrate when tested for the presence of diguanide by the method of Gage and Rose (*loc. cit.*) gave a negative result. Crystallisation of the solid from very dilute hydrochloric acid gave N-p-*chlorophenylguanyl*-O-*ethyl*-N′-iso*propyliso*urea *hydrochloride*, m. p. 175° (Found: C, 49·4; H, 6·5; N, 17·8. $C_{13}H_{19}ON_4Cl,HCl$ requires C, 48·9; H, 6·3; N, 17·6%). The hydrochloride when warmed with dilute sodium hydroxide for a few minutes gave an oil which was extracted with benzene, and the extract dried (Na_2SO_4) and evaporated. Crystallisation of the residue from light petroleum (b. p. 80—100°) gave the *base* (IX; R = Cl, R′ = Prβ, R″ = OEt) as colourless

needles, m. p. 100—101° (Found: C, 54·9; H, 6·7; N, 19·6. $C_{13}H_{19}ON_4Cl$ requires C, 55·2; H, 6·7; N, 19·8%). The *acetate*, prepared from the base in acetone solution with acetic acid, had m. p. 149° (Found: C, 52·4; H, 6·7; N, 16·8. $C_{13}H_{19}ON_4Cl,CH_3{\cdot}CO_2H$ requires C, 52·6; H, 6·7; N, 16·4%).

(*b*) *N*-*p*-Chlorophenylguanyl-*S*-methyl-*N'*-*iso*propyl*iso*thiourea (2 g.) and alcoholic ammonia (40 c.c.) were heated in a sealed tube for 6 hours at 120°. The contents of the tube were evaporated to dryness to leave an oil which was treated with 2N-hydrochloric acid. Part dissolved, leaving a residue of the hydrochloride of *N*-*p*-chlorophenylguanyl-*O*-ethyl-*N'*-*iso*propyl*iso*urea which was removed by filtration. The filtrate was made faintly alkaline to brilliant-yellow with ammonia, and N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide hydrochloride, m. p. and mixed, m. p. 244°, was precipitated.

Reaction of N-p-*Chlorophenylguanyl*-S-*methyl*-N'-iso*propyl*iso*thiourea with* iso*Propylamine*.—(IX; R = Cl, R' = Pr^{β}, R'' = SMe) (1 g.) and *iso*propylamine (10 c.c.) were heated for 6 hours at 120° in a closed vessel. Evaporation of the contents left an oil which was extracted with 2N-hydrochloric acid. The extract was filtered and treated with ammonia so as just to destroy the Congo-red acidity. On standing, a precipitate developed (0·7 g.) which was collected and proved to be N^1-*p*-chlorophenyl-N^4 : N^5-di*iso*propyldiguanide hydrochloride. Crystallised from water it formed colourless prisms, m. p. and mixed m. p. 254°.

Condensation of Dimethylcyanamide and Thiourea.—Dimethylcyanamide (7·0 g.) was added to a suspension of thiourea (7·6 g.) in boiling butanol (20 c.c.) and the mixture boiled for 1 hour. On cooling, the resulting solution deposited crystals which were filtered off, washed with butanol, and dried (2·3 g., m. p. 160—162°). Concentration of the mother liquors gave a further crop (2 g.) of the same material of rather lower m. p. The two crops were combined and crystallised from methanol to give *NN*-dimethylthiourea as colourless prisms, m. p. 163—164°, undepressed by an authentic specimen (Found: S, 31·0. Calc. for $C_3H_8N_2S$: S, 30·8%). Wallach (*Ber.*, 1899, **32**, 1873) gives m. p. 158—159°. The final butanol mother liquors were evaporated to dryness under reduced pressure, leaving a brown viscous oil which when treated with methanolic picric acid afforded dimethylguanidine picrate, m. p. 226—228° undepressed in admixture with an authentic specimen (Clarke and Phillips, *J. Amer. Chem. Soc.*, 1923, **45**, 1755).

Guanylthiourea.—Pellets of potassium hydroxide (168 g.) and carbon disulphide (100 c.c.) were added to a well-stirred suspension of dicyandiamide (126 g.) in acetone (1 l.) cooled to − 10° to − 5°. The temperature was then allowed gradually to rise to 15°, and the resulting suspension stirred at 15—25° for a further 3¼ hours. The yellow product was filtered off, washed with acetone, and dried (288 g.).

This dipotassium ω-cyanoguanidinodithiocarbonate (118 g.) was dissolved in water (4 l.), and acetic acid added with good stirring until the solution was neutral to litmus. The precipitated 4 : 6-diamino-2-thio-1 : 3 : 5-thiadiazine was filtered off, washed with water, and dried (43 g.); m. p. > 300°.

4 : 6-Diamino-2-thio-1 : 3 : 5-thiadiazine (40 g.), water (500 c.c.), and orthophosphoric acid (37·5 c.c.) were boiled under reflux for 4 hours. The charcoaled and filtered solution was allowed to cool overnight, and guanylthiourea phosphate crystallised; it was collected, washed with water, and dried (yield, 27·5 g.). A further small crop (4 g.) was obtained by evaporation of the mother liquors to small bulk. To a solution of this phosphate (50 g.) in water (500 c.c.) kept at 90° a solution of hydrated barium hydroxide (80 g.) in water (100 c.c.) was added until the solution was faintly alkaline to phenolphthalein. After cooling, the barium phosphate was filtered off and the filtrate evaporated under reduced pressure to small bulk. The *base*, which crystallised, was collected, dried, and crystallised from methanol; it formed colourless prisms, m. p. 175—176° (decomp.) (Found: N, 47·2. $C_2H_6N_4S$ requires N, 47·45%).

p-*Chlorophenylguanylthiourea* (VIII; R = Cl, R' = H).—*p*-Chlorophenyldicyandiamide (78 g.) and a methanolic solution of hydrogen sulphide (800 c.c., saturated at 0°), containing dissolved sodium (1·6 g.), were heated in a closed vessel at 70—80° for 50 hours. The resulting mixture was filtered and poured into a mixture of water (1600 c.c.) and hydrochloric acid (80 c.c.). The small amount of material precipitated was filtered off, washed, and crystallised from alcohol–water, and consisted of p-*chlorophenyldithiobiuret* (4 g.), m. p. 163—165° (decomp.) undepressed by an authentic sample (see below) (Found: Cl, 14·4; S, 26·6. $C_8H_8N_3ClS_2$ requires Cl, 14·5; S, 26·1%). The acid filtrate from this material was evaporated under reduced pressure to 500 c.c. and cooled. The p-*chlorophenylguanylthiourea hydrochloride* [colourless plates from dilute hydrochloric acid, m. p. 190—191° (decomp.) (Found: Cl, 26·4; S, 12·1. $C_8H_9N_4ClS,HCl$ requires Cl, 26·8; S, 12·1%)], which separated was filtered off, dissolved in hot water (1 l.), and the solution poured into water (100 c.c.) and ammonia (30 c.c., *d* 0·88) to give the *base* as a tar which gradually solidified. It was collected, washed well with water, and crystallised from methanol–water, giving colourless prisms (66 g.), m. p. 166—167° (Found: C, 42·2; H, 4·0; N, 24·5; Cl, 15·6; S, 14·4. $C_8H_9N_4ClS$ requires C, 42·0; H, 3·9; N, 24·5; Cl, 15·5; S, 14·0%).

N-p-*Chlorophenylguanyl*-S-*methyl*iso*thiourea* (IX; R = Cl, R' = H, R'' = SMe).—*p*-Chlorophenylguanylthiourea (92 g.), methanol (50 c.c.), and methyl iodide (10 c.c.) were boiled under reflux for 4 hours and the solution then evaporated to dryness under reduced pressure. Trituration of the oily residue with ethyl acetate gave N-p-*chlorophenylguanyl*-S-*methyl*iso*thiourea hydriodide* which crystallised from acetone–benzene as colourless prisms (10·7 g.), m. p. 167° (Found: C, 29·5; H, 3·1; S, 8·7. $C_9H_{11}N_4ClS,HI$ requires C, 29·15; H, 3·2; S, 8·6%).

N-p-*Chlorophenylguanyl*-S-*ethyl*iso*thiourea* (IX; R = Cl, R' = H, R'' = SEt).—Prepared similarly from *p*-chlorophenylguanylthiourea and ethyl iodide, the *hydriodide* crystallised from acetone–benzene as colourless plates, m. p. 179—180° (Found: C, 31·3; H, 3·6; N, 14·5; S, 8·7. $C_{10}H_{13}N_4ClS,HI$ requires C, 31·2; H, 3·6; N, 14·6; S, 8·3%).

N-p-*Chlorophenylguanyl*-S-iso*propyl*iso*thiourea* (IX; R = Cl, R' = H, R'' = SPr^{β}).—A suspension of *p*-chlorophenylguanylthiourea (4·6 g.) in dry alcohol (25 c.c.) was boiled under reflux with *iso*propyl bromide (5 c.c.) for 24 hours. A clear solution was gradually obtained from which crystalline material separated towards the end of the reaction. After cooling, the resulting N-p-*chlorophenylguanyl*-S-iso*propyl*iso*thiourea hydrobromide* was filtered off and crystallised from water containing a little hydrobromic acid; it formed colourless prisms, m. p. 223° (decomp.) (Found: C, 37·9; H, 4·6; S, 9·4; Br', 22·9. $C_{11}H_{15}N_4SCl,HBr$ requires C, 37·6; H, 4·6; S, 9·1; Br', 22·8%).

N-p-*Chlorophenylguanyl*-S-*benzyl*iso*thiourea* (IX; R = Cl, R' = H, R'' = $S{\cdot}CH_2Ph$).—*p*-Chlorophenylguanylthiourea (22·8 g.), methanol (150 c.c.), and benzyl chloride (11·5 c.c.) were boiled under

reflux for 2 hours. The crystalline *hydrochloride* which separated on cooling was collected, and the mother liquors evaporated to give a second crop; crystallised from methanol it formed colourless plates, m. p. 226—227° (decomp.) (Found: C, 51·0; H, 4·6; S, 9·4. $C_{15}H_{15}N_4ClS,HCl$ requires C, 50·7; H, 4·5; S, 9·0%).

p-*Chlorophenyldithiobiuret* (Experiments by Dr. N. Barton).—(*a*) Sodium (6·9 g.) was dissolved in alcohol (150 c.c.). To this solution, cooled in ice, finely ground *S*-methyl*iso*thiourea sulphate (44 g.) was added with stirring. After ½ hour a solution of *p*-chlorophenyl *iso*thiocyanate (51 g.) in alcohol (100 c.c.) was added. The mixture was allowed to regain room temperature and stirred for 2 hours. During this time a solid separated which was isolated by dilution with water (600 c.c.) and filtration, washed with water, and dried. Crystallisation from benzene gave 1-p-*chlorophenyl*-4-*S*-*methyliso*dithiobiuret (63 g.), m. p. 135—137° (Found: C, 42·1; H, 3·9; N, 16·4; S, 24·6. $C_9H_{10}N_3ClS_2$ requires C, 41·6; H, 3·9; N, 16·2; S, 24·7%).

Sodium (9·2 g.) was dissolved in alcohol (400 c.c.), and hydrogen sulphide passed in for 2 hours. 1-*p*-Chlorophenyl-4-*S*-methyl*iso*dithiobiuret (52 g.) was added and the solution refluxed for 1 hour. After filtering and cooling, the solution was acidified with acetic acid and diluted with water to a total volume of 1 l. The solid which separated was filtered off, washed with water, and crystallised from dilute alcohol to give *p*-chlorophenyldithiobiuret, m. p. 164° (decomp., varies with rate of heating) (Found: S, 26·1. Calc. for $C_8H_8N_3ClS_2$: S, 26·1%) (yield, 65%). Repeated recrystallisation of *p*-chlorophenyldithiobiuret frequently leads to decomposition and is to be avoided. The substance is soluble in cold dilute sodium hydroxide, in ammonia, and in dilute sodium carbonate solution on warming.

(*b*) *p*-Chloroaniline (19 g.) and perthiocyanic acid (15 g.) (Chattaway and Stevens, *J.*, 1897, **71**, 607) were ground together and then stirred and heated by means of a boiling water-bath. After about ½ hour the fluid melt solidified. After a further ½ hour's heating the melt was ground and refluxed with carbon disulphide for ½ hour. The undissolved solid was collected, washed with a little carbon disulphide, and dried. It was then dissolved in 2N-sodium hydroxide at 40—50°, the solution filtered to remove insoluble matter, and the filtrate acidified with 2N-hydrochloric acid. The precipitated solid was filtered off, washed with water until acid-free, and crystallised from dilute alcohol to give *p*-chlorophenyldithiobiuret (11·3 g.), m. p. 163—165° (decomp.) undepressed by material made by method (*a*).

p-*Iodophenylguanylthiourea* (VIII; R = I, R′ = H).—*p*-Iodophenyldicyandiamide (42·9 g.) (Part XXVIII, *loc. cit.*) was heated in a pressure bottle with a solution of hydrogen sulphide (10 g.) in methanol (300 c.c.) at 70—80° for 48 hours. After cooling, the mixture was made strongly acid to Congo-red with hydrochloric acid. The crystalline material which separated on standing was collected and crystallised from methanol to give p-*iodophenylguanylthiourea hydrochloride* as colourless plates, m. p. 196—197° (decomp.) (Found: C, 26·6; H, 2·8; N, 15·8; S, 9·2. $C_8H_9N_4IS,HCl$ requires C, 26·9; H, 2·8; N, 15·7; S, 9·0%).

iso*Propylguanylthiourea* (XI; R = Pr$^\beta$, R′ = H).—N^1-*iso*Propyldicyandiamide (12·6 g.) (Part XXVIII) and a saturated (at 0°) solution of hydrogen sulphide in methanol were heated in a pressure bottle at 70—80° for 48 hours. The resulting solution was evaporated to dryness under reduced pressure, leaving an oil which crystallised on stirring with water and standing, and was almost pure iso*propylguanylthiourea* (9 g.). Crystallised from water it formed colourless prisms, m. p. 115—116° (Found: C, 37·8; H, 7·3; N, 35·1; S, 20·4. $C_5H_{12}N_4S$ requires C, 37·5; H, 7·5; N, 35·0; S, 20·0%). Addition of oxalic acid to the liquors from the above base precipitated the oxalate which afforded a further quantity of the base (2·5 g.) on decomposition with alcoholic ammonia.

N-iso*Propylguanyl*-S-*methyliso*thiourea (XII; R = Pr$^\beta$, R′ = H, R″ = Me).—When methyl iodide (3·5 g.) and a solution of *iso*propylguanylthiourea (4·0 g.) in acetone (20 c.c.) were mixed, reaction occurred with evolution of heat. After 1 hour the filtered mixture was evaporated to small bulk and allowed to crystallise. The *hydriodide* (5·65 g.) was filtered off and crystallised from acetone–benzene as colourless plates, m. p. 156—157° (Found: C, 24·2; H, 5·0; S, 10·6. $C_6H_{14}N_4S,HI$ requires C, 23·8; H, 5·0; S, 10·6%).

N-*Butylguanylthiourea* (XI; R = Bu$^\alpha$, R′ = H), prepared from N^1-*n*-butyldicyandiamide and hydrogen sulphide as described above for the *iso*propyl compound, crystallised from water as colourless prisms, m. p. 144—145° (Found: C, 41·2; H, 7·8; N, 32·3; S, 18·7. $C_6H_{14}N_4S$ requires C, 41·4; H, 8·0; N, 32·2; S, 18·4%). When treated with methyl iodide in acetone solution as in the preparation of *S*-methyl-*N*-*iso*propylguanyl*iso*thiourea it was converted into *N*-*n*-butylguanyl-*S*-methyl*iso*thiourea hydriodide which failed to crystallise.

NN-*Diethylguanylthiourea* (XI; R = R′ = Et), prepared from N^1 : N^1-diethyldicyandiamide and hydrogen sulphide, separated from water as colourless prisms, m. p. 118—120° (Found: C, 41·5; H, 7·9; N, 32·0; S, 18·6. $C_6H_{14}N_4S$ requires C, 41·4; H, 8·0; N, 32·2; S, 18·4%).

N-(NN-*Diethylguanyl*)-S-*methyliso*thiourea *hydriodide* (as XII; R = R′ = Et, R″ = Me), prepared from the preceding compound and methyl iodide, crystallised from acetone–benzene as colourless plates, m. p. 133—134° (Found: C, 26·9; H, 5·4; S, 10·2. $C_7H_{16}N_4S,HI$ requires C, 26·6; H, 5·4; S, 10·1%).

N-*Methyl*-N-iso*propylguanylthiourea* (XI; R = Me, R′ = Pr$^\beta$), prepared from N^1-methyl-N^1-*iso*propyldicyandiamide, crystallised from water as colourless prisms, m. p. 139—140° (Found: C, 41·3; H, 7·8; N, 32·3; S, 18·2. $C_6H_{14}N_4S$ requires C, 41·4; H, 8·0; N, 32·2; S, 18·4%).

N-(N-*Methyl*-N-iso*propylguanyl*)-S-*methyliso*thiourea (XII; R = R″ = Me, R′ = Pr$^\beta$), prepared from (XI; R = Me, R′ = Pr$^\beta$) and methyl iodide, separated from acetone–benzene as colourless rectangular plates, m. p. 183—185° (Found: C, 26·8; H, 5·3; S, 10·5. $C_{17}H_{16}N_4S,HI$ requires C, 26·6; H, 5·4; S, 10·1%).

Attempted Reaction of Methyl iso*Propylcyanamide with* S-*Methyliso*thiourea *in Acetone.*—Sodium (2·3 g.) was dissolved in acetone (100 c.c.), the solution cooled, and *S*-methyl*iso*thiourea sulphate (14 g.) and methyl*iso*propylcyanamide (9·8 g.) added. The mixture was stirred overnight and the bulk of the solvent removed by evaporation on the steam-bath. The solid product was filtered off, washed with acetone, and extracted with warm methanol. On evaporation to small bulk, the extract deposited crystalline material which was collected and recrystallised from methanol (yield, 2·65 g.); colourless plates, m. p. 222° (decomp.) (Found: C, 41·9; H, 7·0; N, 32·5; S, 18·5. $C_6H_{12}N_4S$ requires C, 41·9;

H, 7·0; N, 32·6; S, 18·6%). From the analysis the substance appeared to be 2-*amino*-4-*methylthio*-6-*dimethyldihydro*-1 : 3 : 5-*triazine* (XIII), and this was supported by the formation of the same substance in approximately the same yield in two separate experiments in which (*a*) dimethylcyanamide was employed in place of methyl*iso*propylcyanamide, and (*b*) no cyanamide was used.

The above compound (2 g.), *p*-chloroaniline hydrochloride (1·7 g.), and water (5 c.c.) were boiled under reflux for 18 hours. Unreacted *p*-chloroaniline was removed by distillation in steam, and the solution filtered from insoluble matter. Addition of the filtrate to sodium hydroxide precipitated a tarry solid which gradually solidified. This was collected, washed with water, and crystallised from 50% alcohol to give a compound which was presumed to be 2-*amino*-4-p-*chloroanilino*-6-*dimethyldihydro*-1 : 3 : 5-*triazine* (XIV), m. p. 130—131° undepressed by the material described below.

Condensation of p-*Chlorophenyldiguanide with Acetone.*—*p*-Chlorophenyldiguanide hydrate (32·5 g.) (Part IV, *loc. cit.*), acetone (130 c.c.), and piperidine (2 c.c.) were refluxed for 15 hours, cooled, treated with decolourising carbon, and filtered. The filtrate was poured into water (280 c.c.), allowed to stand for 2 hours, and the solid collected, washed with water, and crystallised from aqueous alcohol to give a product (believed to be 2-amino-4-*p*-chloroanilino-6-dimethyldihydro-1 : 3 : 5-triazine) as colourless prisms (yield, 13 g.), m. p. 130—131° (Found : C, 49·2; H, 5·4; N, 26·1. $C_{11}H_{14}N_5Cl,H_2O$ requires C, 49·0; H, 5·2; N, 26·0%). The substance loses water at its m. p., and recrystallisation of the resulting material from aqueous alcohol affords the original hydrate. It does not form a copper complex with ammoniacal copper sulphate.

Reaction of p-*Chlorophenylguanylthiourea with* iso*Propylamine in Presence of Mercuric Oxide.*—(*a*) *p*-Chlorophenylguanylthiourea (23 g.) was dissolved in methanolic *iso*propylamine (200 c.c. of 32·5%), and the solution stirred with mercuric oxide (43 g.) for 48 hours at room temperature. It was then heated at 40—50° for 2 hours, cooled, and filtered. The filtrate was evaporated to dryness and the residue extracted twice with a mixture of water (50 c.c.) and 2N-hydrochloric acid (20 c.c.) at 60—65° for a few minutes and filtered cold. The residue was washed well with water, dried, and crystallised from methanol to give *p*-chlorophenyldicyandiamide (yield, 14·5 g., 74·5%), m. p. 203° undepressed by an authentic specimen. The above acid extracts were combined and added to sodium hydroxide solution to give a tarry precipitate which was collected, heated with 5% acetic acid at 90—100°, and then cooled. The insoluble material which was collected by filtration consisted of unchanged starting material (14·5%). The acetic acid extract was made alkaline with sodium hydroxide and extracted with benzene. The dried benzene extract was evaporated to dryness; a little acetic acid was added to the residue, and the excess removed by evaporation. On stirring the residue with acetone, a small amount of material remained undissolved which was identified as N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide acetate, m. p. and mixed m. p. 184—185° (yield, <0·5%).

(*b*) A suspension of *iso*propylamine hydrochloride (3·2 g.), *p*-chlorophenylguanylthiourea (6·9 g.), and mercuric oxide (6·5 g.) in nitrobenzene (20 c.c.) was stirred at 130—135° for 18 hours. After cooling the mixture was stirred with 2N-hydrochloric acid (40 c.c.) for 1 hour and filtered from mercuric sulphide. The acid layer was separated, and the nitrobenzene re-extracted with 2N-hydrochloric acid (20 c.c.). The combined acid extracts were freed from nitrobenzene by extraction with benzene, clarified by filtration through kieselguhr, and made alkaline (brilliant-yellow) with ammonia. The precipitated product was filtered off and crystallised from water; m. p. 243—244° undepressed in admixture with the hydrochloride of N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (yield, 2 g.).

Reaction of p-*Chlorophenyldicyandiamide with* iso*Propylamine at Low Temperatures.*—*p*-Chlorophenyldicyandiamide (19·5 g.) in methanol (200 c.c.) containing *iso*propylamine (65·0 g.) was stirred at room temperature for 48 hours and then at 40—50° for 2 hours. The solution was then evaporated to dryness and extracted with 5% acetic acid (50 c.c.) at 50—60°. The insoluble material was unchanged *p*-chlorophenyldicyandiamide (18·4 g.). The acetic acid extract was poured into excess of sodium hydroxide solution and extracted with benzene. Evaporation of the dried benzene solution left a small quantity of oil which was dissolved in ether and the solution neutralised with acetic acid. On evaporation and lixiviation of the residue with acetone, a small amount of a substance was obtained which was identified as N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide acetate, m. p. 184—186° (yield, <0·5%).

A similar experiment performed with the addition of mercuric oxide (43 g.) gave a rather higher yield (2·5%) of diguanide.

Reaction of p-*Chlorophenylguanylthiourea with Mercuric Oxide.*—*p*-Chlorophenylguanylthiourea (1·15 g.), mercuric oxide (2·2 g.), and methanol (15 c.c.) were heated under reflux for 20 hours, and the mixture then filtered. Acidification of the filtrate with hydrochloric acid and dilution with water gave *p*-chlorophenyldicyandiamide (yield, 0·35 g.), m. p. and mixed m. p. 200—201°.

Reaction of p-*Iodophenylguanylthiourea with* iso*Propylamine.*—*p*-Iodophenylguanylthiourea hydrochloride (7·1 g.), *iso*propylamine (1·2 g.), and mercuric oxide (4·3 g.) were heated in nitrobenzene at 130—135° for 18 hours with stirring. The cooled mixture was stirred with 2N-hydrochloric acid and then filtered. The two layers were separated, and the nitrobenzene layer extracted again with 2N-hydrochloric acid. The combined acid extracts were shaken with benzene, separated, and made faintly alkaline (brilliant-yellow) with ammonia. The precipitated product crystallised from water to give N^1-*p*-iodophenyl-N^5-*iso*propyldiguanide hydrochloride (2·1 g.) (Part XXVIII), m. p. and mixed m. p. 237—239°.

Reaction of iso*Propylguanylthiourea with Mercuric Oxide.*—*iso*Propylguanylthiourea (2·0 g.) and mercuric oxide (6·0 g.) were stirred with a 25% solution of trimethylamine in alcohol (20 c.c.) for 18 hours at 50—60°. The resulting suspension was filtered, and the filtrate evaporated to small bulk and cooled. The crystalline material which separated was collected and crystallised from aqueous methanol; m. p. 203—208° [Found : C, 37·9; H, 6·5; N, 34·9; *M* (Rast), 155. Calc. for $C_5H_{10}N_4S$: C, 38·0; H, 6·35; N, 35·4%; *M*, 158], and was considered to be a mixture of two or more of the several possible oxidation products of *iso*propylguanylthiourea. The filtrate from this material was evaporated to dryness and dissolved in dioxan. On standing, crystals of *iso*propyldicyandiamide separated; m. p. 113—115° undepressed in admixture with authentic material ($\frac{1}{2}C_4H_8O_2$) (Part XXVIII).

Reaction of iso*Propylguanylthiourea with* p-*Bromoaniline.*—*p*-Bromoaniline (8·6 g.) was dissolved in

2N-hydrochloric acid (25 c.c.), and *iso*propylguanylthiourea (8 g.) and mercuric oxide (11 g.) added. After 1 hour's stirring the mixture was boiled under reflux for a further hour, then acidified with 2N-hydrochloric acid and cooled. To precipitate any soluble mercury a little sodium sulphide was added and the mixture filtered. The filtrate was neutralised with ammonia, and the precipitated product filtered off and crystallised from water. It had m. p. 243—244°, identical with the N^1-*p*-bromophenyl-N^5-*iso*propyldiguanide hydrochloride previously described (Part XXVIII).

Reaction of p-*Chlorophenylguanyl*-S-*methylisothiourea with Alkylamines.*—(a) *Methylamine.* (i) In methanol. *p*-Chlorophenylguanyl-*S*-methyl*iso*thiourea hydriodide (2·6 g.) was added to a cooled methanolic methylamine solution (19 c.c. of 13%), the temperature being kept below 10°. After 4 days at room temperature the solution was evaporated to dryness and the residue treated with N-hydrochloric acid (20 c.c.) at 50—60°. After cooling, filtration left a residue which was identified as *p*-chlorophenyldicyandiamide, m. p. and mixed m. p. 202° (0·25 g.). The filtrate was poured into excess of sodium hydroxide to give an oil which was isolated by extraction with benzene followed by evaporation of the benzene extract. It was converted into the hydrochloride by suspending in warm water, neutralising with 2N-hydrochloric acid and allowing the filtered solution to cool. The product which crystallised (0·25 g.) was identified as N^1-*p*-chlorophenyl-N^5-methyldiguanide hydrochloride (Part X), m. p. and mixed m. p. 227—228°.

(ii) In aqueous methanol. *p*-Chlorophenylguanyl-*S*-methyl*iso*thiourea hydriodide (1 g.) was dissolved in methanol (15 c.c.) and aqueous methylamine (20 c.c. of 21%) added gradually with cooling (temperature below 20°). The mixture was left for 6 days at room temperature and then evaporated to dryness. Acid extraction of the residue with cold 2N-hydrochloric acid afforded no detectable diguanide. The acid-insoluble material was pure *p*-chlorophenyldicyandiamide (0·45 g.), m. p. and mixed m. p. 201—202°.

(b) *Dimethylamine.* The hydriodide of (IX; R = Cl, R′ = H, R″ = SMe) (2·6 g.) was added to methanolic dimethylamine (14 c.c. of 24%), the temperature being kept below 10°, and the mixture left for 4 days. Evaporation of the solution to dryness gave a residue which was completely soluble in N-hydrochloric acid. This acid solution was poured into excess of sodium hydroxide, to give a precipitate of almost pure N^1-*p*-chlorophenyl-N^5 : N^5-dimethyldiguanide (yield, 90%), m. p. 168—169° undepressed by an authentic specimen (Part X).

(c) iso*Propylamine.* (i) *p*-Chlorophenylguanyl-*S*-methyl*iso*thiourea hydriodide (1 g.) was dissolved in methanol (10 c.c.), and *iso*propylamine added below 20°. After 6 days the mixture was evaporated to dryness, and the residue extracted with cold 2N-hydrochloric acid (10 c.c.), leaving undissolved *p*-chlorophenyldicyandiamide (yield, 20%), m. p. and mixed m. p. 200—201°. The acid extract was poured into excess of sodium hydroxide solution to give an oily precipitate which gradually solidified. This material was collected, washed with water, dried, and dissolved in a small quantity of acetone. Addition of an acetone solution of acetic acid to destroy the alkalinity to brilliant-yellow gave a precipitate of N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide acetate (yield, 20%), m. p. and mixed m. p. 184—185°.

(ii) *p*-Chlorophenylguanyl-*S*-methyl*iso*thiourea hydriodide (1·85 g.) was mixed into a thin paste with *iso*propylamine (0·5 g.) and water (1 c.c.), and the mixture heated to 130° during 1 hour under an air condenser. After a further 15 hours at 130—135°, the melt was boiled for a short time with 2N-sodium hydroxide, cooled, and extracted with ether. The ether extract was in turn extracted with 2N-acetic acid, the extract basified with sodium hydroxide, and the liberated base extracted with ether. This ether extract was shaken with 2N-hydrochloric acid, and the acid extract separated and made faintly alkaline (brilliant-yellow) with ammonia to give N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide hydrochloride, m. p. and mixed m. p. 243—244° (yield, 70%).

(d) *Methyl*iso*propylamine.* *p*-Chlorophenylguanyl-*S*-methyl*iso*thiourea hydriodide (1 g.) dissolved in methanol (10 c.c.) was treated with methyl*iso*propylamine (4 c.c.), the mixture being kept below 20°. After 6 days the mixture was worked up by evaporation and extraction with cold 2N-hydrochloric acid to give *p*-chlorophenyldicyandiamide (0·3 g.) and an acid-soluble portion. The base obtained from the latter was converted by means of acetic acid in acetone into its acetate (0·05 g.) which was identical with N^1-*p*-chlorophenyl-N^5-methyl-N^5-*iso*propyldiguanide acetate (Part X), m. p. and mixed m. p. 212—213°.

Condensation of N-iso*Propylguanyl*-S-*methylisothiourea Hydriodide with* p-*Chloroaniline.*—*p*-Chloroaniline (1·3 g.), the hydriodide of (XII; R = Pr$^\beta$, R′ = H, R″ = Me) (3·0 g.), and water (5 c.c.) were refluxed for 18 hours. Unreacted *p*-chloroaniline was removed by steam distillation, leaving the hydriodide of N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (needles from water, m. p. 170—171°) which was converted into the base by dissolution in water and basification with sodium hydroxide. The base, isolated by ether extraction, afforded the hydrochloride of (III; R = Pr$^\beta$, R′ = H), m. p. and mixed m. p. 243—244° (yield, 30%).

Condensation of N-iso*Propylguanyl*-S-*methylisothiourea Hydriodide with* p-*Anisidine.*—*p*-Anisidine (1·2 g.) and (XII; R = Pr$^\beta$, R′ = H, R″ = Me) hydriodide (3·0 g.) were boiled with water (5 c.c.) for 18 hours. The mixture was diluted with water (15 c.c.) and extracted with ether. The aqueous layer was made alkaline to Clayton-yellow with sodium hydroxide, and the resulting oil extracted with ether. The ethereal solution was neutralised with 2N-hydrochloric acid; crystals of N^1-*p*-methoxyphenyl-N^5-*iso*propyldiguanide hydrochloride (forthcoming publication) separated, m. p. and mixed m. p. 230—231° (yield, 47·5%).

Condensation of N-(N-*Methyl*-N-iso*propylguanyl*)-S-*methylisothiourea Hydriodide with* p-*Chloroaniline.*—*N*-(*N*-Methyl-*N*-*iso*propylguanyl)-*S*-methyl*iso*thiourea hydriodide (3·2 g.), *p*-chloroaniline (1·3 g.), and water (5 c.c.) were refluxed for 20 hours. The resulting mixture was made faintly alkaline (brilliant-yellow) with ammonia, and unreacted *p*-chloroaniline removed by steam distillation. On cooling, the hydriodide of N^1-*p*-chlorophenyl-N^5-methyl-N^5-*iso*propyldiguanide separated (m. p. 224° after crystallisation from water) (yield, 13%); it was identified by conversion into the corresponding acetate (Part X), m. p. and mixed m. p. 212°.

Condensation of N-n-*Butylguanyl*-S-*methylisothiourea Hydriodide with* p-*Bromoaniline.*—*p*-Bromoaniline (8·0 g.) was added to an aqueous solution of an equivalent quantity of crude *N*-*n*-butylguanyl-*S*-methyl*iso*thiourea hydriodide (see above) in water (25 c.c.), and the solution boiled under reflux for

22 hours. Ammonia was added to make the solution faintly alkaline to brilliant-yellow, followed by steam distillation to remove unreacted *p*-bromoaniline. After dilution with water to 80 c.c., hydrochloric acid was added until the mixture was acid to Congo-red. A little insoluble matter was removed by filtration, and the filtrate added to excess of sodium hydroxide solution. The precipitated base was extracted with benzene, and the product re-extracted from the benzene as its hydrochloride by shaking with 2N-hydrochloric acid. Addition of ammonia to this acid extract precipitated N^1-*p*-bromophenyl-N^5-*n*-butyldiguanide hydrochloride (first prepared by another route to be described in a forthcoming publication), m. p. and mixed m. p. 210°.

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335. *Decomposition of DDT* [1 : 1 : 1-*Trichloro*-2 : 2-*di*-(4-*chlorophenyl*)-*ethane*] *by Basic Substances.*

By K. A. LORD.

The reaction between DDT and some basic substances has been investigated at 30°. It has been shown that the rate of decomposition of DDT by methylamine is dependent upon the square of the amine concentration.

CONSIDERATION of the structure of DDT suggests that it will contain highly reactive chlorine atoms in the aliphatic part of the molecule, and the first recorded reaction of DDT is that of the elimination of hydrogen chloride from the molecule by alcoholic caustic alkali (Zeidler, *Ber.*, 1874, **7**, 1180). This reaction gives 1 : 1-dichloro-2 : 2-di-(4-chlorophenyl)ethylene, which is ineffective as an insecticide : it is therefore important to know the conditions under which it will occur, especially as it has formed the basis of a theory to account for the insecticidal activity of DDT (Martin and Wain, *Nature*, 1944, **154**, 512). In addition, at the time this work began, it seemed possible that DDT might form addition compounds with amines, and that this might account for its insecticidal activity. Preliminary experiments, however, showed that ammonia and simple amines decomposed DDT at room temperature to give the ethylenic compound. These reactions were therefore investigated more thoroughly. There was no evidence of the loss of further chlorine from the molecule, although other workers (Sheibley and Prutton, *J. Amer. Chem. Soc.*, 1940, **62**, 840; Cristol and Haller, *ibid.*, 1945, **67**, 2222; Martin and Wain, *Analyst*, 1947, **72**, 1) have described the removal of more chlorine than this from both DDT and its analogues.

Reaction of DDT with Amino-compounds.—The reactions between DDT, ammonia, and the methylamines in aqueous dioxan solution have been investigated. The reaction rate appears to run parallel with the amount of ionisation to be expected from the dissociation constants of the amines. It seems likely, therefore, that the reaction takes place between DDT and the amine ion, a conclusion borne out by the results with a series of other organic substances, some of which are of considerable biological importance, wherein it was found that only those which ionise to give an alkaline solution decompose DDT.

It can be shown that the reaction rate of DDT with both mono- and di-methylamine is proportional to the concentration of DDT. The reaction rate, however, depends on the *square* of the concentration of methylamine and is not directly proportional to it as might be expected from the investigations of Cristol (*J. Amer. Chem. Soc.*, 1945, **67**, 1494) on the decomposition of DDT by sodium hydroxide in alcoholic solution. This was confirmed by similar experiments with ethylene glycol monoethyl ether as solvent, showing the result not to be due to a specific solvent effect.

The dependence of the reaction rate on the square of the amine concentration can be explained by postulating either the formation of a DDT–amine complex or the reaction of DDT with a dimerised form of the amine.

The reaction between DDT and methylamine can be expressed stoicheiometrically by the equation

$$(C_6H_4Cl)_2CH{\cdot}CCl_3 + NH_2{\cdot}CH_3 \longrightarrow (C_6H_4Cl)_2C{:}CCl_2 + NH_2{\cdot}CH_3,HCl$$

and it has been shown experimentally that the rate of reaction $= k[DDT][NH_2{\cdot}CH_3]^2$.

The reaction taking place with the formation of a complex may be represented :

Stage I. $DDT + NH_2{\cdot}CH_3 \longrightarrow$ Complex
Stage II. $Complex + NH_2{\cdot}CH_3 \longrightarrow (C_6H_4Cl)_2C{:}CCl_2 + NH_2{\cdot}CH_3,HCl + NH_2{\cdot}CH_3$

From stage I, $[\text{Complex}] \propto \frac{[\text{DDT}][\text{NH}_2\cdot\text{CH}_3]}{k_1}$

From stage II, Rate of reaction $= k_2[\text{Complex}][\text{NH}_2\cdot\text{CH}_3]$

Thus the rate of reaction $= k[\text{DDT}][\text{NH}_2\cdot\text{CH}_3]^2$.

If reaction with the amine dimer is assumed, it can be shown that, if most of the amine is present as monomer, the dimer concentration is proportional to the square of the amine concentration, thus giving the equation for rate of reaction in accordance with experimental results. The arguments are applicable to the methylamine ion ($NH_3\cdot CH_3^+$) as well as to the un-ionised amine. The reaction of DDT with an amine dimer seems unlikely under the conditions used.

Attempts to prepare compounds of various amines and DDT were not successful, and mixed melting-point curves of DDT with tribromoaniline, β-naphthylamine, benzamide, and acetanilide did not show compound formation; these results are in agreement with those of Martin and Wain (*loc. cit.*).

The reaction of carbon tetrachloride with simple aliphatic amines has recently been suggested to us as a possible model for this DDT decomposition.

Effect of Medium on Reaction Rate.—The reaction rate of DDT with aqueous sodium hydroxide is greatly affected by the reaction medium, the order of reaction with various DDT solvents being:

Ethylene glycol monoethyl ether > acetone > dioxan > benzene (= 0)

With alcoholic sodium hydroxide the reaction rates are in the same order but much higher, and the reaction rate in benzene is not zero. (Analogous effects are found with strong organic bases, *e.g.*, nicotine and methylamine.)

Catalytic Effect of Metals.—Catalysis of the thermal decomposition of DDT by metals or metallic oxides has been reported by a number of workers (Balaban and Sutcliffe, *Nature*, 1945, **155**, 755; Fleck and Haller, *J. Amer. Chem. Soc.*, 1944, **66**, 2095). In the course of this work the decomposition of DDT by sodium hydroxide was shown to be catalysed by a number of metallic salts.

Effect of Degree of Alkalinity of Solutions.—It has been shown that, with all reagents in one phase, buffer solutions ranging from pH 7 to 11 decompose DDT, and in general the speed of reaction increases with increasing pH; some decomposition was noticeable at a pH as low as 7·1.

Experimental.

5 Ml. aliquots of DDT solution were measured into stoppered bottles at 30°; when thermal equilibrium had been attained 1 ml. portions of the appropriate reagent solution were added and the reaction allowed to proceed. The mixture was then acidified with 50% (8N) nitric acid, and excess of N/50-silver nitrate solution added, followed by 1 ml. of nitrobenzene and 5 drops of saturated ferric ammonium alum as indicator. The excess of silver was then titrated with ammonium thiocyanate (approx. N/50). When a substance such as nicotine, possessing an emulsifying effect, was present the precipitated silver chloride was filtered off before the titration. In a number of cases the results have been expressed as the fraction of DDT decomposed or the ratio of ionic chlorine found to the initial amount of DDT.

Order of Reaction of DDT with Methylamine.—The reaction of DDT with methylamine can be represented by the equation

$$(C_6H_4Cl)_2CH\cdot CCl_3 + NH_2\cdot CH_3 = (C_6H_4Cl)_2C{:}CCl_2 + NH_2\cdot CH_3,HCl$$

If one reactant is in excess and the concentration of the other is c, the observed order of reaction n will be determined by the number of molecules of the reactant not in excess, and may be calculated by the differential method suggested by van't Hoff.

Thus $$-\mathrm{d}(c-x)/\mathrm{d}t = k(c-x)^n$$

and hence $$n \log (c-x) = \log k$$

which represents a straight line with slope n.

Table I.

2N-Ammonia (1 ml.) and 2N-DDT (5 ml.) in dioxan or benzene.

Time (days).	Cl′/DDT (dioxan).	Cl′/DDT (benzene).	Time (days).	Cl′/DDT (dioxan).	Cl′/DDT (benzene).
0·0	0·00	0·00	5·78	0·14	0·00
1·75	0·05	0·00	7·81	0·16	0·00
3·72	0·10	0·00	9·83	0·17	0·02
			12·80	0·25	0·02

TABLE II.

2N-Methylamine (1 ml.) and 2N-DDT (5 ml.) in dioxan.

Time (hours).	Cl′/DDT.	Time (hours).	Cl′/DDT.	Time (hours).	Cl′/DDT.	Time (hours).	Cl′/DDT.
0·00	0·00	9·50	0·28	23·58	0·51	51·13	0·71
1·67	0·06	11·25	0·31	26·83	0·54	63·25	0·79
3·25	0·11	13·83	0·35	31·00	0·55	72·00	0·81
5·08	0·17	16·00	0·40	40·50	0·66	87·83	0·86
7·50	0·22	20·67	0·46	43·50	0·64	92·75	0·89
						98·50	0·88

TABLE III.

2N-Dimethylamine (1 ml.) and 2N-DDT (5 ml.) in dioxan.

Time (hours).	Cl′/DDT.	Time (hours).	Cl′/DDT.	Time (hours).	Cl′/DDT.	Time (hours).	Cl′/DDT.
0·00	0·01	10·67	0·28	39·83	0·67	69·58	0·82
2·00	0·08	15·25	0·37	45·75	0·70	74·08	0·85
4·00	0·13	15·50	0·38	50·17	0·72	78·17	0·85
5·67	0·17	23·42	0·48	55·83	0·77	87·67	0·89
7·75	0·22	29·75	0·56	63·75	0·82	95·50	0·90

TABLE IV.

2N-Trimethylamine (1 ml.) and 2N-DDT (5 ml.) in dioxan or benzene.

Time (days).	Cl′/DDT (dioxan).	Time (days).	Cl′/DDT (dioxan).	Time (days).	Cl′/DDT (benzene).	Time (days).	Cl′/DDT (benzene).
0·00	0·00	7·00	0·24	0·0	0·00	5·0	0·02
0·13	0·00	9·00	0·22	1·0	0·01	8·0	0·03
2·00	0·07	12·00	0·25	2·0	0·00	11·0	0·04
4·08	0·12	15·13	0·28				

TABLE V.

0·44N-Methylamine (1 ml.) and 0·4N-DDT (5 ml.) in dioxan.

Initial concn. of DDT (*a*) = 0·333 g.-mol./l.
" " methylamine (*b*) = 0·073 g.-mol./l.

Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).
0·00	0·000	16·08	0·023	54·17	0·047	87·50	0·051
2·50	0·005	18·50	0·023	64·00	0·046	92·08	0·052
3·00	0·006	21·75	0·027	64·20	0·047	94·42	0·053
4·92	0·008	30·08	0·033	69·50	0·051	96·00	0·054
8·25	0·015	40·10	0·039	81·00	0·052	112·00	0·055
		45·75	0·042			117·08	0·059

TABLE VI.

0·53N-Methylamine (1 ml.) and 0·10N-DDT (5 ml.) in dioxan.

Initial concn. of DDT (*a*) = 0·083 g.-mol./l.
" " methylamine (*b*) = 0·088 g.-mol./l.

Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).
0·00	0·000	15·07	0·011	53·35	0·026	92·68	0·036
2·03	0·002	20·35	0·013	63·10	0·030	98·73	0·037
4·53	0·003	23·98	0·015	68·35	0·030	114·85	0·040
6·72	0·005	29·25	0·017	76·87	0·032	135·93	0·043
9·50	0·007	39·12	0·022	—	0·035	143·82	0·043
		44·37	0·023			149·73	0·046
		48·12	0·024				

TABLE VII.

0·53N-Methylamine (1 ml.) and 0·10N-DDT (5 ml.) in ethylene glycol monoethyl ether.

Initial concn. of DDT (*a*) = 0·083 g.-mol./l.
" " methylamine (*b*) = 0·088 g.-mol./l.

Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).
0·00	0·000	5·85	0·011	18·08	0·023	52·22	0·040
2·00	0·005	11·28	0·017	25·55	0·028	71·35	0·042
3·40	0·007	12·33	0·018	34·65	0·031	72·25	0·043
3·43	0·007			47·73	0·040		

TABLE VIII.

0·53N-Methylamine (1 ml.) and 0·075N-DDT (5 ml.) in ethylene glycol monoethyl ether.

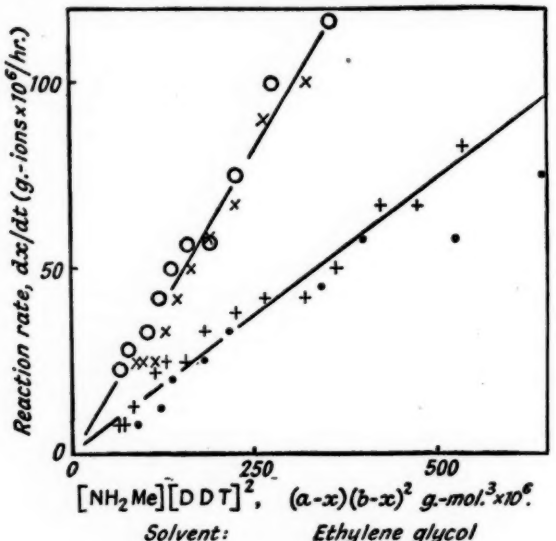
Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).	Time (hours).	Concn. of Cl′ (*x*).
0·00	0·000	16·25	0·017	45·75	0·029	64·40	0·035
1·68	0·004	22·17	0·021	50·80	0·031	69·87	0·036
4·17	0·006	24·25	0·021	54·55	0·031	71·43	0·035
9·37	0·012	40·10	0·028	64·03	0·036	77·10	0·036

The reaction of DDT with methylamine in dioxan was followed with the amine in excess (Table II), and the reaction rate and concentration of DDT at various times determined. The order of reaction obtained by the method described above, log (reaction rate) being plotted against log (DDT concentration), was shown to be 1.

Similarly from the data in Table III it was shown that the reaction rate of dimethylamine with DDT is dependent upon the concentration of DDT.

The results in Table V show that the rate of reaction was dependent on the square of the free amine concentration.

Reaction of DDT with Methylamine.



Thus the rate of reaction of DDT with methylamine in water–dioxan (1 : 5) solution can be expressed by the equation

$$\text{Reaction rate} = dx/dt = k(a - x)(b - x)^2 = k[\text{DDT}][\text{amine}]^2.$$

Estimates of dx/dt plotted against those of $(a - x)(b - x)^2$ obtained from the data in Tables V and VI show a linear relationship and confirm this finding.

Similar estimates obtained from data on the reaction taking place in water–ethylene glycol monoethyl ether (1 : 5) (Tables VII and VIII) show that the same relationship holds in this medium (see figure).

Action of Various Substances on DDT Solutions at 30°.—A solution of DDT in ethylene glycol monoethyl ether was decomposed by aqueous solutions of sodium carbonate, guanidine carbonate, calcium hydroxide, and nicotine. Urea, glycine, lecithin, and creatinine had no apparent action. An aqueous solution of choline decomposed DDT in dioxan solution; sodium taurocholate and deoxycholate appeared to have a slight action, but not acetylcholine or ox bile.

Effect of Medium on the Decomposition of DDT in Solution by Sodium Hydroxide at 30°—The percentage decomposition caused by 1 ml. portions of aqueous sodium hydroxide added to 5 ml. of 0·10N-DDT in various solvents is summarised below.

Solvent.	Time (hours).	Percentage decomposition: 40% NaOH.	8% NaOH.
Benzene	25	1, 2	1, 1
Dioxan	20	4, 4, 3, 3	44, 46
Acetone	1	6, 7	32, 37, 37, 36
Ethylene glycol monoethyl ether...	1	92, 97	99, 98

The lower decomposition by 40% sodium hydroxide is probably due to the separation of the materials into two phases.

The action of 2N-alcoholic sodium hydroxide was almost complete in 1 minute in ethylene glycol monoethyl ether or acetone. The benzene solution showed 69—72% decomposition in 30 minutes. The decomposition of the dioxan solution after one minute was 34—47%, after two minutes 61—69%, after five minutes 93%, and after 30 minutes 100%.

Catalytic Effect of Metals.—0·1 Ml. of aqueous solutions of ferrous sulphate, ferric ammonium alum, copper sulphate, and manganese sulphate each containing 1% of metal, added to 5 ml. portions of 0·05N-DDT in acetone subsequently treated with 1 ml. of N-sodium hydroxide, caused increased decomposition. The percentage decomposition after one hour was: Fe^{**}, 81; Fe^{***}, 86; Cu, 86; Mn, 85; metal absent, 65.

A similar effect was observed in the case of an ethylene glycol monoethyl ether solution of DDT treated with nicotine. The percentage decomposition after 23 days in each case was: Fe^{**}, 20; Fe^{***}, 22; Cu^{**}, 39; Mn, 9; Al^{***}, 28; metal absent 14.

Effect of pH.—10 Ml. aliquots of N/50-DDT in ethylene glycol monoethyl ether were mixed with 2 ml. portions of buffer solutions shown below. The decomposition occurring was observed at various time intervals.

Buffer solutions.	pH.	Percentage decomposition. 7 days.	23 days.
4M/10-KH_2PO_4 + 3N/10-NaOH	7·1	2	12
4M/10-H_3PO_4 + 3N/10-NaOH	9·6	6	95
4M/10-$NaHCO_3$ + 3N/10-NaOH	10·0	19	92
4M/10-Na_2HPO_4 + 3N/10-NaOH	10·9	5	85
4M/10-$NaHCO_3$	8·0	12	56
4M/10-Na_2CO_3	10·2	28	100

I wish to acknowledge the advice and encouragement of Dr. F. Tattersfield throughout the investigation, and also the suggestions made by Dr. A. E. Alexander as to the possible mode of reaction between DDT and alkaline amino-compounds.

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[*Received, November 20th,* 1947.]

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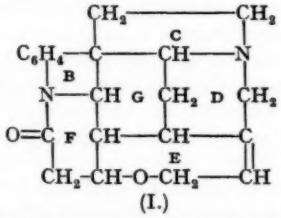
336. *Strychnine. Part III. Some New Reduction Products.*

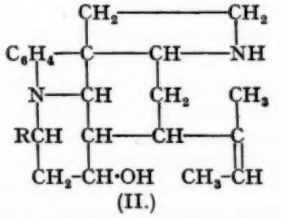
By G. R. CLEMO and T. J. KING.

The reduction of strychnine and three of its derivatives by sodium in liquid ammonia is investigated and partial structures are assigned to the products.

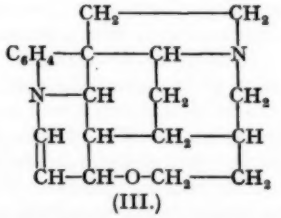
THE emphasis of recent work on the structure of strychnine, particularly in this country, by Robinson and his collaborators, has been upon the relationship of the strychnine and *neo*strychnine double bond to N(*b*), and upon the possible point of attachment of the nitrogen atom to the carbazole residue now almost universally assumed to be present in the alkaloid. The present work has been undertaken with the object of obtaining evidence of the structure of the lactam and ether rings of strychnine. Little work has been done on this point in recent years, and, although early work sufficed for a structure of these rings to be postulated, a more rigorous proof is desirable. The structure generally thought to be the most probable for strychnine is (I). The structures of the compounds described below will be illustrated on the basis of this formulation for convenience and not from conviction that the rings (E and F) are indeed seven- and six-membered respectively.



(I.)



(II.)



(III.)

When one considers the chemistry of the two rings E and F of strychnine (I), it appears that the double bond in ring E and the nitrogen and carbon atoms which form the lactam group interact in a profound way. Thus dihydrostrychnidine is considerably more basic than strychnidine; strychnine is much more readily reduced than strychnidine; and the lactam ring, comparatively readily opened in strychnine, cannot be opened to give dihydrostrychnic acid (Oxford, Perkin, and Robinson, *J.*, 1927, 2389). The distance of the double bond from the

lactam grouping renders explanation of these facts difficult, and, to our knowledge, no satisfactory explanation has been made. A further important reaction, the nature of which is quite obscure, is the change of strychnine to *iso*strychnine. Irrefutable evidence of the structure of rings E and F might throw light on these unsolved problems. We thus consider it is highly desirable to establish (i) the size of the lactam ring, (ii) whether the ether oxygen of strychnine is attached to a secondary or a tertiary carbon atom.

The relationship of the lactam group to the point of attachment of the ethereal oxygen is indicated by the ready hydrolysis of strychninolic acid (Leuchs and Schneider, *Ber.*, 1909, **42**, 2495), and a proof of the secondary nature of the alcohol in a compound such as (II) would virtually prove the six-membered nature of ring F. Conversely, if the hydroxyl group in such a compound should prove tertiary, the lactam ring must be considered as five-membered, and drastic changes made in the proposed formula for strychnine. Work on *iso*nitrososstrychnine (Wieland and Kaziro, *Annalen*, 1933, **506**, 60) does indeed point to the secondary nature of such an alcohol, but the proof is not as rigorous as could be desired.

A fission of ring E in which the oxygen atom remains attached to ring F, followed by oxidation to a ketone, would afford the simplest and most direct proof of the secondary nature of the attachment of the oxygen. Raper and Clemo (*J.*, 1946, 891) have suggested that such a fission occurs when strychnine is reduced at high temperature and pressure in methanol solution over Raney nickel. A hydroxyl group is certainly present in the product (termed Base D), but it is not possible to say with certainty that this is attached to ring F; there seems no activating group which would justify us in assuming that fission would take place preferentially in the desired direction, and fission may have occurred in a manner to separate the oxygen from ring F. The double bond in ring E in strychnine cannot facilitate the fission, as we have shown that quantitative conversion of strychnine into dihydrostrychnine occurs over Raney nickel at 100°/100 atm. long before ring fission can take place. Again, it is possible that, under the conditions of the reaction, a change to the *iso*strychnine series is involved in the production of Base D, as dihydro*iso*strychnine can be prepared by heating dihydrostrychnine to 145° in methanol in the presence of a base (Oxford, Perkin, and Robinson, *loc. cit.*), and the fact that in our hands the drastic reduction of dihydro*iso*strychnine does not give Base D does little to discredit the hypothesis. It thus appeared that unless confirmatory evidence for the structure of Base D could be obtained, the secondary or tertiary nature of its hydroxyl group could have no significance.

The Leuchs degradation indicates that strychnine is an allyl ether, and one very convenient method for the fission of allyl ethers involves reduction by sodium in liquid ammonia in the presence of a suitable proton donor, usually an alcohol. If fission of the ether link in strychnine should occur under these conditions, it would be reasonable to assume that no rearrangement had taken place and that the hydroxyl group in the product would be attached as desired to ring F. We have shown that rearrangement is most unlikely during such a reduction by recovering strychnine, dihydrostrychnine, Base D, and strychnidine from solution of sodium methoxide in liquid ammonia in nearly quantitative yields.

A factor greatly influencing the course and speed of the reduction of strychnine and its derivatives with sodium in liquid ammonia is their low solubility in this solvent, which results in the comparatively slow reaction of sodium with the added alcohol, the speed being comparable to that of dissolution of the base; it also limits the scale upon which the reaction can be carried out, 1—2 g. of the alkaloid being as much as can be successfully reduced using vessels of 100 ml. capacity. Increase of the amount of base used without increase of the amount of solvent leads to the necessity of a wasteful separation of product from unchanged material.

Reduction of strychnine itself, using the amounts of sodium and alcohol necessary for the fission of the ether linkage, was disappointing; in some experiments a small amount of pure product was isolated, but not until at least six mols. of added alcohol and sodium were used could consistent results be obtained. The process described in the experimental section was eventually standardised and proved reliable and satisfactory.

The primary *product* (II, R = OH)—subsequently referred to as Base E—isolated from strychnine was a colourless compound, stable in neutral or alkaline solution, containing at least one atom of active hydrogen, as shown by acetylation and by the Zerewitinoff method: this had been formed by the addition to strychnine of six hydrogen atoms instead of the expected two. On treatment in the cold with mineral acid a yellow solution was obtained which, on basification, gave an amorphous yellow solid of indeterminate melting point which was almost certainly a mixture. Its empirical formula showed, however, that it had been formed from Base E by elimination of water, the resulting double bond presumably being responsible for the colour.

Boiling of Base E with dilute mineral acids brought about still further decomposition, giving a dark red solution from which basification produced only a red resinous material. The colour reactions of this series of compounds resemble those of strychnidine rather than strychnine (*e.g.*, no Otto reaction could be detected), and thus it appeared that the lactam group had been modified.

The reduction of the lactam group and the ether linkage would account for the uptake of four hydrogen atoms, and the presence of two further atoms we attribute to the fission of the allylamino-group also present in the molecule. For this to be true, Base E should contain a secondary amino-group in addition to a hydroxyl group, and should give at least two active hydrogens in a Zerewitinoff determination. In practice, only one active hydrogen could be detected, and only a monoacetyl derivative was obtained on acetylation. This latter evidence is not, however, very significant, as the instability of Base E to acids prevented vigorous acetylation conditions being employed. The low active-hydrogen determination is also discounted as evidence, because two active hydrogen atoms are definitely present in the compound obtained by catalytic reduction of Base E, where the modified lactam group has been removed. Interaction between this group and the hydroxyl group is considered to be responsible for the low active hydrogen content. It is interesting to note that Base D, in which a hydroxyl group is also present, gives no active hydrogen in a Zerewitinoff determination, although the corresponding strychnidine derivative gives one active hydrogen atom.

The reduction of tertiary amides with sodium in ammonia has not been previously reported, and a model experiment was undertaken with *N*-methylacetanilide in the hope that light might be thrown on the nature of the alteration of the lactam group. Acetaldehyde was isolated in fair yield, after treatment of the crude product with acid, as its 2 : 4-dinitrophenylhydrazone. This reaction, constituting a new method of preparation of aldehydes from derivatives of the corresponding acids, was further investigated. However, dialkylamides were not reduced, and experiments with a wide range of anilides were disappointing, the above reduction to give acetaldehyde being the most successful. The reaction may, however, be of use in the hydrolysis of alkylanilides when groups in the remainder of the molecule make the use of strongly acid or alkaline reagents undesirable.

The model experiment left no doubt that Base E contained a potential aldehyde group, and this was confirmed by its ready reaction with hydroxylamine. The presence of an unmodified aldehyde group is inherently unlikely, and the compound exhibited no reducing action or aldehyde colour reactions; the comparative difficulty of reduction of this compound is also against its formulation as an aldehyde. We consider the most likely formulation of Base E to be (II, R = OH) (hydroxytetrahydrodi*chano*strychnidine), that is, an aldehyde ammonia or *pseudo*-base, and the formulation affords an explanation of the acid-catalysed change previously mentioned. Acid treatment would presumably give the true base, as with the *pseudo*-bases of the quinoline series; wandering of the double bond into the five-membered ring might occur, basification leading to further bond migration of an indeterminate nature. A double bond which had so wandered into the central part of the molecule might be expected to be resistant to reduction for purely steric reasons, and so the observation that the yellow base (anhydrohydroxytetrahydrodi*chano*strychnidine) retains its colour and so presumably its double bond on reduction at 150°/100 atm. over Raney nickel adds support to this interpretation. The only alternative to formulation as the *pseudo*-base is formulation as the corresponding anhydro-base. However, this would necessitate Base E having a molecule of water of crystallisation. We have been quite unable to eliminate water from the base without decomposition. Water is in fact evolved quantitatively on melting, but the resulting melt cannot be reconverted into Base E by treatment with water or aqueous solvents. The loss of water is indeed the first result of treating Base E with acetic anhydride; acetylation of the anhydro-base then takes place slowly.

A compound of the structure we attribute to Base E should be oxidisable to the lactam with alkaline ferricyanide, but under the conditions attempted by us no such oxidation could be accomplished. This failure is significant in that it raised doubts as to the validity of the evidence for the existence of the group $>C—N(b)<$ in strychnine, based as it is on the non-oxidation under similar conditions of *pseudo*strychnine.

Reduction of the double bond in Base E, using Adams's catalyst, was unsuccessful. Reduction over Raney nickel at 100°/80 atm., however, reduced both double bond and the *pseudo*-basic hydroxyl group to give a *compound*, which we call hexahydrodi*chano*strychnidine, containing two active hydrogen atoms. This shows no aldehydic properties, nor is it sensitive to acid; it is clearly a derivative of strychnidine.

The reduction of dihydrostrychnine was next attempted and again a colourless *compound* (Base F) was obtained, unstable to acids, with which it formed yellow compounds, almost certainly mixtures. In this case there can be little doubt as to the partial structure of the reduction product. Analysis indicated the presence of one oxygen only, and, as vigorous catalytic reduction produced dihydrostrychnidine, the only acceptable structure is (III), which may be called dehydrodihydrostrychnidine. The occurrence of this compound as the anhydro-base need not decide us against formulating Base E as the *pseudo*-base, as quite small changes in the molecule might be expected to alter the relative stability of *pseudo*-base to *anhydro*-base.

Reduction of Base D gave a *product* having the characteristic acid sensitivity and composition for a *pseudo*-basic structure analogous to that of Base E. The melting point with effervescence was very similar to that of Base E, and both these compounds are probably closely related. That no skeletal changes had taken place during the reduction is shown by the catalytic reduction of the compound to the strychnidine derivative obtained by electrolytic reduction of Base D.

Strychnidine was readily reduced in good yield by the standard technique. The reduction was not complicated by the presence of the amido-grouping, and the structure of the *product* as (II, R = H) is virtually certain. The compound is unchanged by acid and has two active hydrogen atoms. Only a monoacetyl derivative could be obtained, which however still contained active hydrogen, and it is perhaps interesting to note that the hydroxyl group of Base D is very resistant to acetylation. The presence of a secondary amino-group in this compound was demonstrated as follows. Straightforward nitrosation was complicated by the reaction of nitrous acid with the aromatic nucleus, and the crude product did not give a Liebermann colour reaction showing the presence of a *N*-nitroso-group. Nitrosation was thus attempted on the compound prepared by condensation of the base with benzenediazonium chloride, in which the benzene nucleus should be protected from attack. The product from this nitrosation gave a positive Liebermann reaction which was not given when a similar series of reactions was applied to strychnidine itself.

Further reduction of the base proved surprisingly difficult. Reduction at room temperature was unsuccessful, and after attempted reduction over Raney nickel at 100°/100 atm. the starting material was recovered nearly quantitatively. However, more vigorous conditions, *i.e.*, 150°/100 atm., resulted in reduction. The *product*, however, was formed by the addition to the base of six atoms of hydrogen instead of the expected two atoms. The same product was later obtained in good yield by the reduction under the same conditions of Base E.

The empirical formula indicates that either the benzene ring has been saturated or two further rings have undergone fission. It is very unlikely that a benzene ring would be reduced while an isolated double bond was unchanged, and all our present evidence is against such a hypothesis. Thus the new base seems to contain a primary amino-group indicating fission of one ring, insufficient hydrogen then being available for saturation of the benzene ring, and an active hydrogen determination gives a value of three per molecule, a result which could not be explained by a simple reduction of the ring. We must therefore suppose that reduction is accompanied by the fission of two more rings. Present evidence is insufficient for us to formulate this very interesting degradation product, but further work on the constitution of this and of the other new degradation products here described is being pursued.

Experimental.

The reductions in ammonia were carried out in an unsilvered Dewar vessel (100 ml.) without rigorous exclusion of moisture. Melting points are uncorrected.

*Hydroxytetrahydrodi*chano*strychnidine* (Base E) (II, R = OH).—Sodium (*ca.* 100 mg.) was added with stirring to a suspension of finely powdered strychnine (1 g.) in liquid ammonia (80 ml.) containing excess (1 ml.) of ethanol. Similar additions were made each time the blue colour disappeared until the colour from one such addition persisted for 15 minutes (total time of reduction ¾—1 hour). The resulting suspension was cautiously acidified by the addition of ammonium chloride, poured into an open beaker, and allowed to evaporate spontaneously. The dry residue was washed (2N-sodium hydroxide, 50 ml.) collected at the pump, washed with water, dried in a vacuum, and recrystallised from boiling ethyl acetate (*ca.* 100 ml.). The *product* separated as colourless radiating clumps of needles (0·53 g., 53%), very soluble in alcohols, less so in acetone, benzene, and ethyl acetate, insoluble in water and light petroleum, m. p. 165° (efferv.) (Found: C, 74·15; H, 8·15; N, 8·5; active hydrogen 0·96. $C_{21}H_{28}O_2N_2$ requires C, 74·1; H, 8·2; N, 8·2%). The compound did not give the Otto reaction, but gave the colour reactions with oxidising agents characteristic of strychnidine derivatives.

Attempted Ferricyanide Oxidation.—(1) Base E (0·5 g.) suspended in aqueous dioxan (25 ml., 20%) containing potassium ferricyanide (1 g.) and sodium hydroxide (1·5 ml. of 2N), was shaken for 48 hours at room temperature. Filtration gave 0·35 g. of Base E, and dilution of the mother liquors liberated a further 0·05 g. (2) The reaction mixture as in (1) was heated on the steam-bath for 4 hours; dilution and filtration gave 0·45 g. of an impure solid from which 0·1 g. of pure Base E was isolated. No further pure

product could be obtained. A model experiment under similar conditions omitting the ferricyanide produced a similar decomposition.

Oximino-Base E.—Base E (0·34 g., 1 mol.) and hydroxylamine hydrochloride (0·07 g., 1 mol.) in water (20 ml.) were refluxed for 1 hour, excess of sodium carbonate solution was added, and the resulting precipitate collected and dried in a vacuum. The very pale yellow *product* was purified by precipitation with light petroleum (b. p. 60—80°) from benzene, and obtained as a microcrystalline powder (0·16 g., m. p. 122°) (Found: N, 11·2. $C_{21}H_{29}O_2N_3$ requires N, 11·8%). Treatment with acid immediately caused hydrolysis with formation of anhydro-Base E.

Action of Acetic Anhydride on Base E.—(*a*) Dissolution of Base E in acetic anhydride for a short time (15 minutes) followed by basification gave a precipitate, m. p. 170° (decomp.), with the composition of the corresponding *anhydro*-base (Found: C, 77·7; H, 8·35. $C_{21}H_{26}ON_2$ requires C, 78·2; H, 8·1%). The poor quality of the analyses in this series is attributed to the instability of the compounds and to the difficulty of purifying them.

(*b*) Base E (0·1 g.) was added to acetic anhydride (1 c.c.); the solid passed slowly into solution during 1 hour at room temperature. After 4 more hours the solution was added to excess of aqueous sodium hydrogen carbonate. The precipitated *acetyl* derivative did not crystallise from any of the usual solvents and was finally obtained by precipitation with water from its alcoholic solution as a colourless powder, m. p. 175° (decomp.). It was extremely unstable to acids (Found: C, 76·4; H, 8·0. $C_{23}H_{28}O_2N_2$ requires C, 75·8; H, 7·7%). More vigorous conditions lead to decomposition with the formation of yellow compounds.

Anhydro-Base E.—Base E dissolved rapidly in the cold in dilute mineral acids with formation of a yellow solution from which aqueous sodium carbonate precipitated an amorphous yellow solid of indeterminate m. p. *ca.* 280° (decomp.). This was partially purified by precipitation from ethanolic solution with water, but later evidence indicated that it was probably a mixture. Its constitution corresponded to its formation from Base E by loss of water (Found: C, 78·1; H, 7·8. $C_{21}H_{26}ON_2$ requires C, 78·2; H, 8·1%).

Reduction at ordinary pressures and temperatures over Adams's catalyst in acetic acid resulted in a very slow absorption of hydrogen, no definite end point being observed, and the yellow products could not be purified. Reduction at 150°/90 atm. in alcohol over Raney nickel gave a yellow solution, showing that the chromophoric double bond is very resistant to reduction.

*Hexahydrodi*chano*strychnidine.*—Base E (0·235 g.) in ethanol (120 ml.) was stirred with hydrogen at 100°/80 atm. and Raney nickel (0·1 g.) for 2 hours. After removal of the catalyst and solvent the gummy residue was triturated with benzene; the *product* crystallised from ethyl acetate as well-defined colourless prisms, m. p. 247°. It dissolved unchanged in acids and gave colour reactions of the strychnidine type (Found: C, 77·7, 77·3; H, 9·3, 8·9; active hydrogen, 1·96. $C_{21}H_{30}ON_2$ requires C, 77·3; H, 9·2%).

If the temperature of the reduction was allowed to rise much above 100° a small quantity of a higher-melting product was formed (see later).

Dehydrodihydrostrychnidine (*Base F*) (III).—Dihydrostrychnine (prepared by stirring strychnine with hydrogen at 100°/100 atm. and Raney nickel for 15 minutes) was reduced with sodium and liquid ammonia in the same way as strychnine, methanol being added instead of ethanol, by the use of which considerable quantities of dihydrostrychnine were sometimes recovered. The crude *product*, m. p. 246°, isolated as before, was obtained pure (0·65 g., m. p. 254°) by crystallisation from ethyl acetate (Found: C, 78·5; H, 8·0. $C_{21}H_{26}ON_2$ requires C, 78·25; H, 8·1%). The solubility of the crude product varied considerably from preparation to preparation, probably owing to the presence of small quantities of impurities. The crystal form of the compound also varied; although it usually formed massive hexagonal prisms, one preparation yielded a product separating as long colourless leaflets. The m. p., however, was always 254°. The pure product was sparingly soluble in cold acetone, benzene, and ethyl acetate, readily soluble in alcohol, and insoluble in water and light petroleum.

Base F gave yellow solutions with acids; the yellow base obtained on neutralisation of the acid solution of 0·5 g. was dissolved in much benzene and fractionated on an alumina column. The appearance of four diffuse bands demonstrated its complexity.

Further reduction of Base F in liquid ammonia proved impossible, the compound being recovered almost quantitatively.

Base F (0·250 g.) in ethanol (120 c.c.) was stirred with Raney nickel and hydrogen at 100°/90 atm. for 1 hour. After removal of catalyst and solvent, the residue crystallised from ethyl acetate as well-defined colourless glistering tablets (0·165 g., m. p. 212—214° alone and mixed with an authentic specimen of dihydrostrychnidine).

Reduction of Base D.—Base D (1 g.) was treated as in the reductions of strychnine and dihydrostrychnine previously described. The crude product was best purified, although with poor recovery, by crystallisation from a large volume (*ca.* 250 ml.) of ethyl acetate, in which it is only very sparingly soluble. The pure *product* formed colourless needles (34%), decomp. 160°, soluble in alcohols but very sparingly soluble in cold acetone, benzene, and ethyl acetate. The m. p. was not depressed by admixture with Base E, but subsequent reduction showed the individuality of the compound (Found: C, 74·4; H, 8·5. $C_{21}H_{28}O_2N_2$ requires C, 74·1; H, 8·2%). Treatment with acid gave a yellow compound.

The above product (0·250 g.) was stirred in ethanol (120 ml.) with Raney nickel and hydrogen at 100°/90 atm. for 1 hour. Removal of catalyst and solvent left a crystalline residue, purified from benzene, m. p. 217° alone and mixed with the product of the electrolytic reduction product of Base D (cf. Clemo and Raper, *loc. cit.*).

*Tetrahydrodi*chano*strychnidine* (II, R = H).—Strychnidine (1 g.) was reduced with methanol as a proton donor, as in the reduction of the previous strychnine derivatives. The crude *product* crystallised from a small volume of boiling benzene in well-defined rectangular tablets (0·63 g., m. p. 192°, b. p. 200°/14 mm., 63%). The m. p. is depressed by admixture with the dihydro*iso*strychnidine of similar melting point described by Oxford, Perkin, and Robinson (*loc. cit.*) (Found: C, 77·8; H, 8·8; active hydrogen, 2·1. $C_{21}H_{28}ON_2$ requires C, 77·8; H, 8·7%). The compound is readily soluble in alcohols, moderately so in acetone and ethyl acetate, insoluble in light petroleum and water. It crystallises with

benzene of crystallisation in which it dissolves if rapidly heated to about 140°; prolonged drying at 130° or distillation are necessary to remove all traces of solvent. It was recovered unchanged from its solution in acids. The *picrate monohydrate* separated slowly on the addition of water to its ethanolic solution as a microcrystalline powder, m. p. 127° (decomp.) (Found: C, 56·5; H, 5·8. $C_{21}H_{28}ON_2,C_6H_3O_7N_3,H_2O$ requires C, 56·7; H, 5·8%); attempts to dehydrate the salt led to its decomposition.

*Acetyltetrahydrodi*chano*strychnidine*.—This reduction product of strychnidine (0·3 g.) was refluxed for 2 hours with acetic anhydride (10 ml.) containing sodium acetate (0·5 g.). Excess of anhydride was decomposed with water, and the whole neutralised (2N-sodium carbonate). The tarry matter which separated was removed, and the solution basified with ammonia. A colourless amorphous *derivative* (0·26 g.) separated, and after being dried in a vacuum was either crystallised from a small volume of light petroleum (b. p. 60—80°), in which it is very soluble, or sublimed at 190°/14 mm. to form stout colourless needles, m. p. 158—160° (Found: C, 75·2; H, 8·1. $C_{23}H_{30}O_2N_2$ requires C, 75·4; H, 8·3%).

*Decahydrotetra*chano*strychnidine*.—The reduction product of strychnidine (0·5 g.) in ethanol (100 c.c.) was reduced over Raney nickel at 150°/100 atm. for 2 hours. Removal of solvent and catalyst left a gummy residue which crystallised on trituration with ethyl acetate. The crude *product* (0·25 g.) crystallised from ethyl acetate in long silky needles, m. p. 258—260° (Found: C, 75·9, 75·9, 76·8, 76·4; H, 10·2, 10·3, 10·3; active hydrogen, 2·9. $C_{21}H_{34}ON_2$ requires C, 76·4; H, 10·3%). The compound was very soluble in alcohols, less so in acetone and benzene, insoluble in water and light petroleum. When it is treated with nitrous acid a gas is evolved; this is taken to indicate the presence of a primary amino-group. With chromic acid in sulphuric acid or ferric chloride in acid solution an orange-red colour was produced, very much less intense than that obtained from strychnidine under the same conditions.

In the preparation of this compound, Base E can take the place of the strychnidine reduction product without significant alteration in the yield of the decahydro-derivative.

Reduction of N-*Methylacetanilide*.—*N*-Methylacetanilide (1·5 g.) was reduced by sodium (0·6 g., 2 atoms) in liquid ammonia containing excess of ethanol (1 ml.). The dry solid obtained after acidification with ammonium chloride and evaporation of the solvent was treated with 2N-hydrochloric acid (100 ml.). The pale yellow solution smelled strongly of acetaldehyde. One-fifth of it was treated with excess of a saturated solution of 2 : 4-dinitrophenylhydrazine hydrochloride in 2N-hydrochloric acid. The yellow crystalline precipitate (0·250 g., 56%) was identified after recrystallisation as acetaldehyde 2 : 4-dinitrophenylhydrazone (m. p. 167°, not depressed by admixture with an authentic specimen).

Half the original solution was concentrated to 5 ml. under reduced pressure, basified, and the liberated oil isolated with ether and distilled. A nearly quantitative yield of methylaniline was obtained (0·51 g., 96%).

We thank the University of Durham for the award to one of us (T. J. K.) of an Imperial Chemical Industries Fellowship.

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337. *A Study of the Mechanism of Oxidation with Chromic Acid. Part II.*

By RONALD SLACK and WILLIAM A. WATERS.

The rates of oxidation of diphenylmethane and of related hydrocarbons with chromium trioxide in glacial acetic acid solution are extremely rapid at first, but soon slacken and stop almost entirely after a few hours though both hydrocarbon and Cr^{VI} remain in the solution. Benzyl radicals may be concerned in the reaction but must be destroyed rapidly by the oxidising agent.

The cessation of the reaction is brought about by Cr^{III} which removes CrO_3 as the inert CrO_4^- or $Cr_2O_7^-$ anions. Other kations and water exert the same effect, whilst the addition of 2·5% of sulphuric acid results in rapid and complete oxidation even at room temperature.

A SURVEY of attempts to elucidate the mechanism of oxidation of organic compounds by chromium trioxide shows that a general correlation cannot easily be made. Many substances are smoothly and completely oxidised in aqueous solution, but unfortunately, special postulates have often been necessary to explain the idiosyncrasies of individual compounds of the same class. Thus, Snethlage (*Rec. Trav. chim.*, 1940, **59**, 111; 1941, **60**, 199; 1942, **61**, 213) found that, whereas the rates of oxidation of succinic acid and formic acid depended largely on the proportion of mineral acid added, no proportionality could be observed with malonic acid. Similarly, although a bimolecular equation gave fairly good constants for succinic acid, the oxidation of oxalic and adipic acids could not be so expressed. Again, Lucci (*Boll. sci. Fac. Chim. Bologna*, 1940, **1**, 333; 1941, **2**, 165, 176) has shown that many aromatic aldehydes gave second-order constants on oxidation, but the data for the three hydroxybenzaldehydes were represented by first-order equations. Westheimer and Novick's equation (*J. Chem. Physics*, 1943, **11**, 506) for the oxidation of *iso*propanol affords yet a further variety.

In a preliminary survey (Part I) it was suggested by one of us (Waters, *J.*, 1946, 1151) that

the initial attack of chromium trioxide on an organic compound was neutral hydrogen abstraction, and it seemed possible that a rationalisation of the kinetic results might emerge on this basis. Experiments have therefore been made with various aromatic hydrocarbons, particularly diphenylmethane which oxidises at a convenient rate for study. Glacial acetic acid was used as a solvent since it is almost inert under the conditions of reaction and gives homogeneous solutions. The course of all the oxidations was followed iodometrically. The sparing solubility (about 1%) of chromium trioxide in acetic acid limits the range of concentrations and an added complication may arise from the formation of chromoacetic anhydride or its acetate (Pictet and Genequand, *Ber.*, 1903, **36**, 2216).

FIG. 1. FIG. 2.

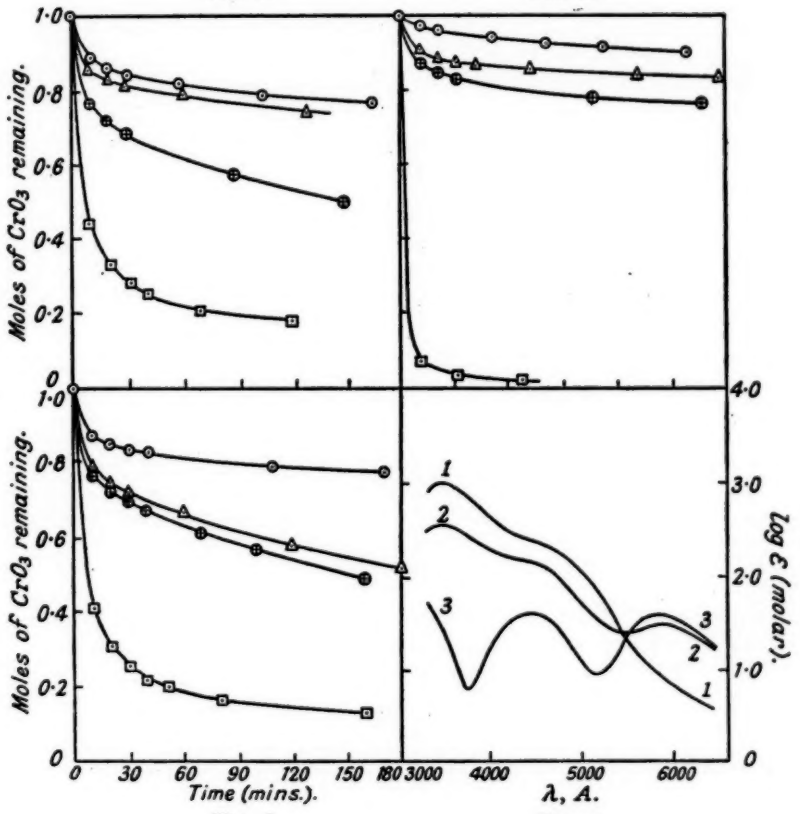

FIG. 3. FIG. 4.

FIG. 1.

⊙ 18°
△ 23°
⊕ 40°
} 1 : ¾, CrO_3 : *diphenylmethane.*
⊡ 40° *Excess diphenylmethane.*

FIG. 2.

⊙ 2 : 1, CrO_3 : *toluene.*
△ 1 : 3, CrO_3 : *diphenylmethane.*
⊕ 1 : 3, CrO_3 : *triphenylmethane.*
⊡ 1 : ¾, CrO_3 : *fluorene.*
Temp. = 30°.

FIG. 3.

⊙ *Benzhydryl acetate.*
△ *Tetraphenylethane.*
⊕ *Diphenylmethane.*
⊡ *Benzhydrol.*

Constant CrO_3. *Oxidant stoicheiometric for benzophenone production. Temp.* = 18°.

FIG. 4.

(1) CrO_3 *in acetic acid.*
(2) *Final reaction mixture*; 1 : ¾, CrO_3 : *diphenylmethane.*
(3) *Fully reduced* CrO_3 *in acetic acid.*

Fig. 1 illustrates the general course of the oxidation of diphenylmethane. Characteristic features of the reaction are : (1) The extraordinarily fast initial rate, which varies in extent with the varying concentrations of the reactants and with temperature. (2) The noticeable decrease in the reaction rate, which finally leads to almost complete cessation of the reaction. (3) The

relatively small effect of temperature. The percentage of oxidising agent finally remaining is approximately constant and does not, within certain limits, depend on the hydrocarbon–CrO_3 ratio (see below—results at 18°):

Molar proportion. CH_2Ph_2.	Molar proportion. CrO_3.	Residual CrO_3, %. (72 hrs.).	Molar proportion. CH_2Ph_2.	Molar proportion. CrO_3.	Residual CrO_3, %. (72 hrs.).
4	4	17	4	2	15
4	3	15	4	1	14·5

Fig. 2 shows the variation in extent of oxidation, different hydrocarbons being used under standard conditions. Whereas difficultly oxidised compounds, *e.g.*, toluene, diphenylmethane, and triphenylmethane, did not reduce all the chromium trioxide in 1—3 days, easily oxidised hydrocarbons, *e.g.*, fluorene, phenanthrene, acenaphthene, and indene, reduced it completely.

The oxidation of diphenylmethane yields mainly benzophenone. No benzhydrol or benzhydryl acetate has been isolated but small amounts of *s*-tetraphenylethane are always found, even during the initial stages. Subsequently, some slight attack at a Ph–C bond takes place and traces of benzoic acid and of phenolic products have been found. We also obtained evidence of the formation of a little succinic acid. Triphenylmethane behaves in a parallel manner and benzophenone and phenol are produced in small yield, the main product in this case being triphenylcarbinol.

A comparison of the rates of oxidation of diphenylmethane, benzhydrol, benzhydryl acetate, and *s*-tetraphenylethane (Fig. 3) reveals that the acetate cannot, in fact, be formed during oxidation since it would accumulate amongst the products. The rate of oxidation of tetraphenylethane is similar initially to that of diphenylmethane, but the ultimate divergence indicates that a steady build-up should occur if (*a*) followed by (*b*) both comprise main steps in the formation of benzophenone:

$$(a)\ CrO_3 + 3CH_2Ph_2 \longrightarrow Cr(OH)_3 + 3Ph_2CH\cdot$$
$$(b)\ 2Ph_2CH\cdot \longrightarrow CHPh_2\cdot CHPh_2$$

The small yield of tetraphenylethane indicates that (*b*) at least is a side reaction. However, the production of this hydrocarbon provides good support for the dehydrogenation theory (Part I), particularly since it has been shown by Nauta and Mulden (*Rec. Trav. chim.*, 1939, **58**, 1070) that dimerisation of the diphenylmethyl radical is one of its characteristic reactions in solution. Again, the detection of succinic acid, evidently an oxidation product of the solvent, supports the free-radical theory (cf. Kharasch *et al.*, *J. Amer. Chem. Soc.*, 1943, **65**, 15; *J. Org. Chem.*, 1945, **10**, 394).

Organic peroxide formation (cf. Part I) has been excluded except as a minor side-reaction, for the comparative rates of oxidation of diphenylmethane in air and in carbon dioxide show only insignificant differences. Hence the sequence

$$(c)\ Ph_2CH\cdot + O_2 \longrightarrow Ph_2CH\cdot OO\cdot$$
$$(d)\ Ph_2CH\cdot OO\cdot + CH_2Ph_2 \longrightarrow Ph_2CH\cdot OOH + Ph_2CH\cdot, \text{ etc.}$$

must be much slower reactions than that of $Ph_2CH\cdot$ with CrO_3. The participation of the kation $Ph_2\overset{+}{C}H$, *e.g.*, viâ the ionic sequence

$$(e)\ CH_2Ph_2 - 2e \longrightarrow Ph_2\overset{+}{C}H + H^+, \text{ etc.}$$

is unlikely since this would yield the stable ester $CHPh_2\cdot OAc$ which could *not* be detected.

A sequence more or less analogous with that suggested for the course of the Étard reaction (Rohde, *Sammlung chem. u. chem. tech. Vorträge*, 1901), involving organic chromium complexes containing Cr^V or Cr^{IV}, does not seem improbable. However, we have found no evidence of the existence of intermediate valency states. These would be expected to have characteristic absorption spectra and our final mixtures disclose only the presence of Cr^{VI} and Cr^{III} (Fig. 4), but these results do not preclude the possibility of disproportionation of Cr^V or Cr^{IV} *in situ*. The relative amounts of Cr^{VI} and Cr^{III} determined from Fig. 4 agree well with the iodometric estimations and with gravimetric estimations of Cr^{VI} as lead chromate.

The Significance of the Chromium Trioxide–Chromic Acid Equilibrium.—The introduction of benzophenone or of a small amount of tetraphenylethane has no pronounced effect on the velocity of oxidation of diphenylmethane, nor is the presence of a *small* amount of water, corresponding to the overall stoicheiometry

$$(f)\ 3CH_2Ph_2 + 2CrO_3 + 6HOAc \longrightarrow 3CHPh_2\cdot OH + 2Cr(OAc)_3 + 3H_2O$$
$$(g)\ 3CH_2Ph_2 + 4CrO_3 + 12HOAc \longrightarrow 3COPh_2 + 4Cr(OAc)_3 + 9H_2O$$

deleterious. However, addition of the Cr^{III} ion or of *anything which serves to convert the oxidising agent in sufficient quantity into* $CrO_4^{=}$ (*or* $HCrO_4^{-}$) *or* $Cr_2O_7^{=}$ *ions has a very marked inhibitory effect.* This phenomenon offers an attractive explanation of the inhibited reactions, for the effective removal of active chromium trioxide, as chromic chromate or dichromate would produce such an effect. This hypothesis receives support from the fact that replacement of the original trioxide by an equivalent amount of either potassium chromate or dichromate virtually eliminates all oxidation under the standard conditions (Fig. 5*a*). Any kation can effect this

FIG. 5*a*. FIG. 6.

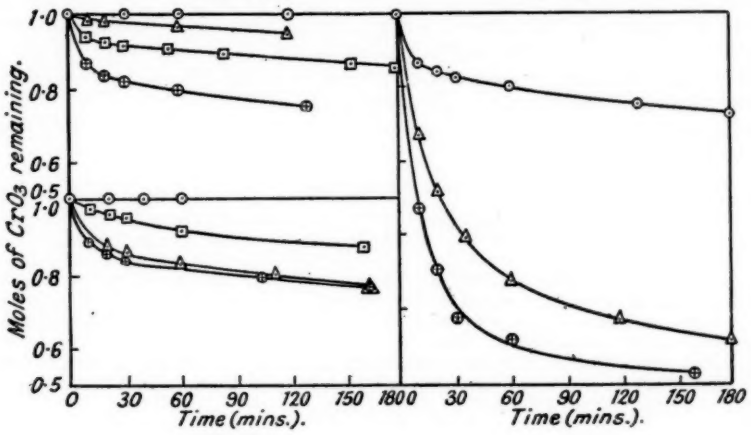

FIG. 5*b*.

FIG. 5*a*.

⊙ *Chromate or dichromate.*
△ Cr^{III} *addition* (2 *molar proportions*).
⊡ Cr^{III} *addition* (1 *molar proportion*).
⊕ 1 : ¾, CrO_3 : *diphenylmethane* (*control*).

FIG. 5*b*.

⊙ 5% *water* = 0·56 *g.-molar.*
⊡ 2·5% *water* = 0·28 *g.-molar.*
△ 1·25% *water* = 0·14 *g.-molar.*
⊕ 1 : 3, CrO_3 : *diphenylmethane* (*control*).

Concentration CrO_3 = 0·005 *g.-molar.* *Temp.* = 18°.

FIG. 6.

⊙ 1 : ¾, CrO_3 : *diphenylmethane* (*control*).
△ *with* $H_2SO_4 : 2H_2O$.
⊕ *with* $H_2SO_4 : H_2O$.
Temp. = 23°.

change. Thus the addition of anhydrous potassium acetate completely inhibits reaction. Similarly, the addition of substances capable of fairly easy dehydration, *e.g.*, orthophosphoric acid, produces identical results. Some slight ultimate attack in the latter case is presumably a measure of the equilibrium

$$(h)\ H_3PO_4 + CrO_3 \rightleftharpoons H_2CrO_4 + HPO_3$$

The change in colour which occurs in an acetic acid solution of chromium trioxide on the addition of any of the above substances is characteristic of the formation of chromate or dichromate; the original opaque dark-red solution at once becomes a transparent orange-yellow. This same colour change, accompanied by a corresponding loss in power of oxidation, is produced by the addition of varying amounts of water, though Fig. 5*b* shows that the equilibria

$$(i)\ CrO_3 + H_2O \rightleftharpoons H_2CrO_4$$
$$(j)\ H_2CrO_4 + CrO_3 \rightleftharpoons H_2Cr_2O_7$$

are decidedly in favour of the dehydrated agent.

Elimination of both the kation and the water effects can be accomplished by the addition of concentrated sulphuric acid to the reaction mixture, and it can be seen from the following table

H_2SO_4, %.	Residual CrO_3, as c.c. of N/10-thiosulphate, after 10 mins.	H_2SO_4, %.	Residual CrO_3, as c.c. of N/10-thiosulphate, after 10 mins.
0	12·4	5·0	0
1·25	7·3	10·0	0
2·5	0·2		

that rapid and complete oxidation occurs in the presence of only 2·5% (by volume). It is significant that chromic sulphate is invariably deposited from the reaction mixture in the illustrative cases. Under these conditions, estimation shows that more than 80% of the hydrocarbon is rapidly converted into ketone despite the fact that some oxidation of the solvent occurs. At low temperatures this side reaction is insignificant, but rapid disruptive oxidation occurs at 100°. Fig. 6 shows clearly that the sulphuric acid and water effects are competitive, though the use of a reagent corresponding to sulphuric acid monohydrate (84% w/w) allows tolerably fast reaction.

Elimination of the water effect can also be achieved in part by the use of an acetic acid–anhydride mixture, but this leads to other peculiarities. Eventually, however, the usual inhibition manifests itself and the reaction does not go to completion. In the presence of acetic anhydride, hot solutions of chromium trioxide in acetic acid are partly reduced, and the formation of succinic acid can clearly be demonstrated.

The investigation is being continued and a more detailed examination of the kinetic problems will be reported.

Experimental.

Materials.—Glacial acetic acid was stabilised by two successive distillations from chromium trioxide; large head and tail fractions were rejected, and acid boiling over a 1° range only was collected for use. All hydrocarbons were distilled or crystallised at least twice. Chromium trioxide was "AnalaR" material dried in a vacuum over sulphuric acid; all other inorganic reagnets were of "AnalaR" quality.

Reaction-velocity Measurements.—Accurately prepared w/v solutions of chromium trioxide and of hydrocarbon in acetic acid were allowed to reach the required temperature in a thermostat, and aliquots were then mixed as rapidly as possible. Samples, withdrawn at known time intervals, were quenched in a standard, previously prepared mixture of potassium iodide and dilute sulphuric acid, and the residual chromium trioxide was estimated iodometrically. To minimise thermal inaccuracies at temperatures greater than 30°, all pipettes were kept within the thermostat.

Investigation of Reaction Products.—(a) *Diphenylmethane.* The reactants mixed as above in several different stoicheiometrical proportions were kept for 24 hrs. at constant temperature. The mixtures were then quenched in acid ferrous sulphate solution and extracted repeatedly with ether. The ethereal extracts were washed with water and all the acidic components were removed by sodium hydroxide extraction. It was found empirically that most of the acetic acid was removed in the first few colourless washings, the acidic oxidation products appearing only in the later, markedly coloured washings. After being dried (Na_2SO_4), the ethereal layer was carefully fractionated, finally under reduced pressure, and gave only diphenylmethane and benzophenone. The bath temperature was never allowed to exceed 190°. The portion which did not distil gave colourless crystals, m. p. 209—210°. Recrystallisation from ligroin raised the m. p. to 211° and this was unchanged by admixture with authentic *s*-tetraphenylethane (Norris *et al.*, *Ber.*, 1910, **43**, 2959). The alkaline extracts were acidified with sulphuric acid, extracted with ether, and divided, by successive sodium hydrogen carbonate and hydroxide washes, into acidic and phenolic portions. After acidification, the former was re-extracted with ether, and the extract evaporated to dryness. The residue was dissolved in boiling ligroin, filtered (charcoal), and allowed to crystallise. This product was sublimed at 100°/5 mm., and the sublimate on crystallisation from water gave white plates, m. p. 119—120°, undepressed by admixture with authentic benzoic acid.

Only traces of phenolic material were obtained from the sodium hydroxide extracts, but the colour produced on coupling with diazotised *p*-nitroaniline was indistinguishable from that of phenol itself when parallel conditions were used. Variations in reaction temperature had no effect on the qualitative results: a slight increase in the yield of acid and phenolic material was observed at 100°. The maximum yield of tetraphenylethane was only of the order of 2%, and that of the other by-products was considerably less than this. A positive colour reaction which indicated the presence of succinic acid was also obtained (see below).

(b) *Triphenylmethane.* The hydrocarbon (22·0 g.) and 1% chromium trioxide solution in acetic acid (300 c.c.) were kept at 30° for 16 hrs. Phenolic, acidic, and neutral fractions were obtained as described above. Triphenylmethane and triphenylcarbinol, m. p. 160—162°, were obtained from the last by fractional crystallisation from alcohol, the final liquors being distilled in a bulb-tube under reduced pressure. Treatment of the distillate with 2 : 4-dinitrophenylhydrazine sulphate in methanol gave orange benzophenone 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 231—232°.

No acidic material was found; traces of crude phenolic material gave, on bromination in aqueous solution, colourless needles, which, crystallised from alcohol, had m. p. 89°. Authentic 2 : 4 : 6-tribromophenol, m. p. 92°, showed no depression on admixture.

Oxidation of Acetic Acid.—A mixture of acetic acid (200 c.c.), acetic anhydride (100 c.c.), and chromium trioxide (50 g.) was heated on the steam-bath until the vigorous, almost uncontrollable reaction had ceased. The initial reaction (at *ca.* 90°) required considerable cooling. When reaction had subsided the bulk of the solvents was removed in a vacuum and the residue treated in a number of ways:

(*a*) The chromium was precipitated from its aqueous acetate solution with dilute ammonia, and the filtrate concentrated after acidifying with hydrochloric acid. A little alcohol was added during this process to destroy unchanged Cr^{VI}. The concentrate was again made alkaline, boiled, filtered, and evaporated. The residual salts gave a strongly positive sulphuric–resorcinol reaction indicative of succinic acid.

(*b*) The aqueous solution of chromic acetate was partly neutralised with ammonia and treated with excess of barium chloride. The resulting precipitate, which contained barium chromate, was warmed with sodium carbonate solution, filtered, and reduced with hydrochloric acid and sodium sulphite. The

solution was made alkaline and filtered, and the filtrate evaporated to dryness. The residue again gave a positive test for succinic acid.

(*c*) Attempted esterification of residual organic acids produced no product which could be identified as ethyl succinate.

More unambiguous results were obtained by the oxidation of acetic acid with chromium trioxide in glacial acetic and sulphuric acids at fairly low temperatures (*ca.* 20°). Much carbon dioxide was evolved throughout, but after dilution with water, continuous ether extraction, removal of the ether, treatment of the residue with ammonia, evaporation to dryness, and distillation with zinc dust the characteristic smell of pyrrole was observed. Its identity was confirmed by means of strongly positive pine-splint tests.

The authors wish to thank Dr. F. B. Strauss for assistance with the spectroscopic measurement; one of them (R. S.) wishes to express his gratitude to the Directors of Messrs. May and Baker Ltd. for granting facilities which have enabled him to participate in this work.

DYSON PERRINS LABORATORY, OXFORD. [*Received, November 22nd,* 1947.]

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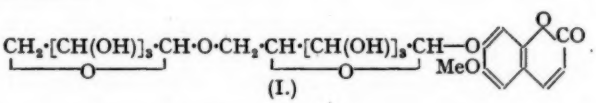
338. *The Syntheses of Glycosides. Part XII. Fabiatrin.*

By D. N. CHAUDHURY, R. A. HOLLAND, and ALEXANDER ROBERTSON.

The syntheses of the β-gentiobioside and the β-primeveroside of 7-hydroxy-6-methoxycoumarin (scopoletin) are described; the latter substance is shown to be identical with the natural glycoside fabiatrin (cf. Merz, *loc. cit.*).

IN the course of their examination of the constituents of *Fabiana imbricata* (Ruiz and Pavon), Edwards and Rogerson (*Biochem. J.*, 1927, **21**, 1010) isolated a glycoside fabiatrin, m. p. 226—228°, which, since it gave 7-hydroxy-6-methoxycoumarin (scopoletin) and glucose on hydrolysis, these authors regarded as a mono-glucoside of the coumarin, a conclusion in keeping with the then accepted view that scopolin, m. p. 215—217°, the known glycoside of scopoletin, contained two molecules of glucose as a disaccharide (see Van Rijin, " Die Glykoside ", 1931, p. 492; Armstrong and Armstrong, " The Glycosides ", 1931, p. 19). After having shown by synthesis that scopolin was the normal mono-β-glucopyranoside of scopoletin, Merz (*Arch. Pharm.*, 1932, **270**, 476), on the basis of Edwards and Rogerson's results and in spite of the considerable divergence in the melting points, concluded that fabiatrin was identical with scopolin. It appeared to us that the latter assumption was unjustified, and when, through the kindness of the late Mr. Rogerson, we were able to examine a small specimen of fabiatrin, it became clear that the two compounds were not identical. After purification we found that fabiatrin had m. p. 236—238°, forming an *acetyl* derivative, m. p. 172°, and in the first instance, accepting the conclusion that glucose was the only sugar formed by the hydrolysis of the glycoside and because the natural phenolic glucosides which have been completely investigated are invariably normal β-pyranosides, we envisaged the possibility of fabiatrin being a β-gentiobioside. Accordingly the O-*hepta-acetyl*-β-*gentiobioside* of scopoletin was synthesised by the quinoline–silver oxide method, and on deacetylation furnished the β-gentiobioside as a *trihydrate*, but comparison of the respective natural and synthetical specimens clearly showed that the two substances were not identical. We therefore re-examined the sugar solution obtained by the hydrolysis of a small amount of fabiatrin, and, finding that it gave the orcinol and phloroglucinol tests for a pentose, we concluded by analogy with other natural biosides that fabiatrin contained (in the following order of preference) either a xylose, a rhamnose, or an arabinose residue in addition to the glucose unit established by Edwards and Rogerson (*loc. cit.*). Consequently the β-primeveroside of scopoletin (I) was next synthesised, being obtained in good yield by the route employed for the corresponding gentiobioside, and was found to be identical in every way with fabiatrin.



(I.)

The identity of the compounds was confirmed by direct comparison of natural and synthetical specimens of the respective *acetyl* derivatives. Fabiatrin and the gentiobioside of scopoletin were obtained as hydrates from which the pure anhydrous substances could not be obtained by drying in a vacuum at elevated temperatures.

Attempts to utilise the general method for the synthesis of biosides (Robertson and Waters,

J., 1931, 1881) in the present series were unsuccessful because we were unable to form the 6-trityl derivative of scopolin by the usual methods.

Experimental.

Scopoletin.—The following synthesis gives better yields than the Perkin method employed by Head and Robertson (*J.*, 1931, 1241). The sodium salt of 2 : 4-dihydroxy-5-methoxybenzaldehyde (from 1 g. of aldehyde, 1·5 g. of sodium hydroxide, and 3 ml. of water) was treated at room temperature with cyanoacetic acid (10 ml. of a solution prepared according to Phelps and Tillotson, *Amer. J. Sci.*, 1908, **26**, 267), and 2 hours later the product was precipitated with hydrochloric acid and hydrolysed by being boiled with 4% hydrochloric acid (50 ml.) for ½ hour. The resulting *scopoletin-3-carboxylic acid* (1·15 g.) separated from warm alcohol in bright yellow needles, m. p. 260—263° after sintering at 250° (Found : C, 55·9; H, 3·7. $C_{11}H_8O_6$ requires C, 55·9; H, 3·4%). In alcoholic solution this acid exhibits an intense blue fluorescence. When a mixture of the acid (1 g.) and ethylene glycol (20 ml.) was kept at 160—170° until evolution of carbon dioxide had ceased and then cooled, scopoletin (0·6 g.) separated in tiny crystals, m. p. 202°, after purification from alcohol. When glycol was replaced by glycerol the evolution of carbon dioxide did not begin below 180° and the product was less pure.

Scopolin.—The following is an improved method for the preparation of the tetra-acetate of scopolin (compare Merz, *loc. cit.*). When scopoletin (1 g.), *O*-tetracetyl-α-glucosidyl bromide (5 g.), silver oxide (4 g.), and quinoline (10 ml.) were mixed, a mild reaction ensued. Next day the solid mixture was extracted with hot acetic acid (75 ml.) and the extract filtered and diluted with water. Crystallised from methyl alcohol, this material gave the tetra-acetate of scopolin in prisms, m. p. 166°, in agreement with Merz (*loc. cit.*). When a specimen of the crude material which had been kept for 2 weeks was crystallised as before, the tetra-acetate was obtained in flat plates, m. p. 184—185°. Since on deacetylation according to the directions of Merz (*loc. cit.*) both forms gave rise to scopolin, m. p. 217—219° (Merz gives m. p. 215—217°), they are regarded as being dimorphic.

β-Gentiobioside of Scopoletin.—Silver oxide (3·2 g.) was well mixed with a paste of scopoletin (1 g.), quinoline (10 ml.), and *O*-hepta-acetyl-α-gentiobiosidyl bromide (5·5 g.) and the mixture well stirred for 15 minutes. Next day the product was extracted with warm acetic acid (100 ml.) and filtered (charcoal), and the filtrate diluted with water (800 ml.). The solid (4·75 g.) was collected, well washed with water, dried, and crystallised from methyl alcohol and then alcohol, giving the 7-*O*-*hepta-acetyl-β-gentiobioside* of scopoletin in colourless needles, m. p. 190°, $[\alpha]_D^{20°}$ − 32° in chloroform (*c*, 0·43) (Found : C, 53·2; H, 5·5. $C_{36}H_{42}O_{21}$ requires C, 53·3; H, 5·2%).

The acetate (3·75 g.) was deacetylated with methyl alcoholic ammonia (500 ml.) at 0° in the course of 16 hours, and, after removal of the ammonia and the solvent under reduced pressure, a solution of the semi-solid residue in the minimum volume of water at 70° was diluted with 4 times its volume of warm alcohol. On cooling, the solution deposited the β-gentiobioside of scopoletin as a *trihydrate* in colourless needles, m. p. 158—162°, after repeated purification from aqueous methyl and ethyl alcohol, $[\alpha]_D^{20°}$ − 150° in water (*c*, 0·4) (Found : C, 46·1; H, 5·7. $C_{32}H_{48}O_{14},3H_2O$ requires C, 46·3; H, 5·9%).

Fabiatrin (1).—Interaction of scopoletin (1·5 g.), hexa-acetyl-α-primeverosidyl bromide (Zemplen, *Ber.*, 1939, **72**, 49) (7 g.), quinoline (10 ml.), and silver oxide (5 g.), followed by isolation of the product with the aid of acetic acid (60 ml.), gave the *hexa-acetate* of fabiatrin which separated from methyl alcohol in colourless, slender needles (4 g.), m. p. 173°, $[\alpha]_D^{20°}$ − 47° in chloroform (*c*, 0·6) (Found : C, 53·7; H, 5·2. $C_{33}H_{40}O_{19}$ requires C, 53·5; H, 5·4%). Deacetylation of this acetate (2·5 g.) with methyl alcohol (175 ml.) saturated with ammonia at 0° gave fabiatrin which, on crystallisation from dilute methyl alcohol, was obtained as a *dihydrate* in rosettes of needles, m. p. 236—238°, $[\alpha]_D^{20°}$ − 140° in water (*c*, 0·5) (Found : C, 48·8; H, 5·5. $C_{21}H_{36}O_{13},2H_2O$ requires C, 48·5; H, 5·7%). On being dried in a high vacuum at 100° the dihydrate lost only part of its water of crystallisation.

A specimen of fabiatrin dihydrate from natural sources was found to melt at 226—228° but on recrystallisation from dilute alcohol had m. p. 236—238°, and was identical with synthetical material (Found : C, 49·0; H, 5·7%). Acetylation of the natural compound (0·1 g.) with acetic anhydride (3 ml.) and sodium acetate (0·5 g.) on the water-bath for 2 hours gave the hexa-acetate which separated from methyl alcohol in slender needles, m. p. 172°, undepressed on admixture with a synthetical specimen (Found : C, 53·7; H, 5·2%).

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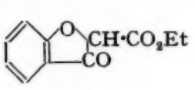
339. *Analogues of Rotenone and Related Compounds. Part II. Coumarono-(2′ : 3′ : 3 : 4)-coumarins.*

By H. I. King, R. H. Holland, F. P. Reed, and Alexander Robertson.

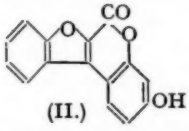
A number of coumarono-(2′ : 3′ : 3 : 4)-coumarins have been synthesised by the condensation of β-ketonic esters (type I) and *m*-dihydroxyphenols according to von Pechmann's method. The indolocoumarin (III) has been prepared by the same procedure.

In pursuit of the objective defined in Part I (*J.*, 1936, 423) we have synthesised by a general method a series of coumarono-(2′ : 3′ : 3 : 4)-coumarins, type (II), which are closely analogous to

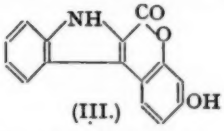
the chromeno-(2′ : 3′ : 3 : 4)-coumarins previously described; as an example, the condensation of the ester (I) with resorcinol according to the method of von Pechmann gave rise to *7-hydroxycoumarono-(2′ : 3′ : 3 : 4)-coumarin* (II). When the ester (I) was replaced by methyl



(I.)



(II.)



(III.)

3-hydroxyindole-2-carboxylate, the *indolocoumarin* (III) was obtained. Attempts to condense esters (type I) with phenol or the cresols by the general method gave negative results or only traces of the desired products.

Experimental.

7-Hydroxycoumarono-(2′ : 3′ : 3 : 4)-coumarin (II). (With W. H. Davis.)—A mixture of ethyl β-coumaranone-2-carboxylate (Friedländer, *Ber.*, 1899, **32**, 1868) (6·8 g.) and resorcinol (12 g.) in 85% sulphuric acid (90 c.c.) was kept at room temperature for 2 days and treated with ice–water. The *product* was repeatedly triturated with much water and then with a little alcohol, and crystallised from a large volume of hot alcohol or acetic acid, forming tiny prisms (7·2 g.), m. p. 311° (decomp.) (Found: C, 71·4; H, 3·4. $C_{15}H_8O_4$ requires C, 71·4; H, 3·4%). Prepared by the pyridine method, the *acetate* formed slender prisms, m. p. 235—236°, from ethyl acetate (Found: C, 69·5; H, 3·7. $C_{17}H_{10}O_5$ requires C, 69·5; H, 3·7%). Methylation of the hydroxycoumarin by the potassium carbonate–acetone method gave the *methyl ether* which separated from much methanol in needles, m. p. 186—187° (Found: C, 72·3; H, 3·5. $C_{16}H_{10}O_4$ requires C, 72·2; H, 3·8%).

7 : 8-*Dihydroxycoumarono-(2′ : 3′ : 3 : 4)-coumarin*.—A solution of ethyl β-coumaranone-2-carboxylate (2 g.) and pyrogallol (2 g.) in methanol saturated with hydrogen chloride at room temperature slowly deposited the coumarin (1·1 g.), m. p. 274—276°, having a green ferric reaction, which could not be economically purified and was converted into the *diacetate*. This derivative formed colourless, elongated, rectangular prisms, m. p. 228—231°, from acetic acid (Found: C, 64·7; H, 3·4. $C_{19}H_{12}O_7$ requires C, 64·8; H, 3·4%). The *dimethyl ether* separated from acetic acid in elongated plates, m. p. 210·5—211·5° (Found: C, 69·1; H, 4·3. $C_{17}H_{12}O_5$ requires C, 68·9; H, 4·1%).

5 : 7-Dihydroxycoumarone-(2′ : 3′ : 3 : 4)-coumarin (hydrogen chloride method) was crystallised from nitrobenzene and then from aqueous glycol, forming tiny prisms, m. p. above 300°, which have a negative ferric reaction. On methylation the compound gave the *dimethyl ether* which separated from acetic acid in colourless needles, m. p. 267—268° (Found: C, 68·9; H, 4·0. $C_{17}H_{12}O_5$ requires C, 68·9; H, 4·1%).

7-Hydroxy-5′-methylcoumarono-(2′ : 3′ : 3 : 4)-coumarin.—When a solution of ethyl 5-methyl-β-coumaranone-2-carboxylate (Auwers, *Annalen*, 1913, **393**, 366) (1 g.) and resorcinol (1 g.) in methyl alcohol was slowly saturated at room temperature with hydrogen chloride, a yellow solid gradually separated. Two days later the mixture was heated on the steam-bath for ½ hour, and on cooling the resulting *coumarin* (0·6 g.) was collected, washed, and crystallised from alcohol, forming needles which have a negative ferric reaction, m. p. above 300° (Found: C, 72·0; H, 3·8. $C_{16}H_{10}O_4$ requires C, 72·2; H, 3·8%). The *acetate* separated from alcohol in long slender needles, m. p. 233° (Found: C, 70·0; H, 3·9. $C_{18}H_{12}O_5$ requires C, 70·1; H, 3·9%).

5 : 7-*Dihydroxy-5′-methylcoumarono-(2′ : 3′ : 3 : 4)-coumarin*.—Prepared by the hydrogen chloride method this substance, m. p. > 300°, could not be economically obtained pure for analysis and was converted into the *diacetate* which formed tiny needles, m. p. 218°, from acetic acid, sparingly soluble in organic solvents (Found: C, 66·0; H, 4·1. $C_{20}H_{14}O_7$ requires C, 65·6; H, 3·9%).

7 : 8-*Dihydroxy-5′-methylcoumarono-(2′ : 3′ : 3 : 4)-coumarin* separated from alcohol in needles, m. p. above 300°, which give a green coloration with alcoholic ferric chloride (Found: C, 68·1; H, 3·7. $C_{16}H_{10}O_5$ requires C, 68·1; H, 3·6%). The *diacetate* formed colourless needles, m. p. 267°, from acetic acid (Found: C, 65·4; H, 3·9. $C_{20}H_{14}O_7$ requires C, 65·6; H, 3·9%).

Ethyl 7-Methyl-β-coumaranone-2-carboxylate.—Interaction of ethyl 2-hydroxy-*m*-toluate (10 g.), ethyl bromoacetate (20 g.), and potassium carbonate (15 g.) in boiling acetone (75 c.c.) for 13 hours gave *ethyl 3-carbethoxy-o-tolyloxyacetate* (15 g.), b. p. 184°/16 mm., m. p. 39°, after purification by distillation 3 times in a vacuum (Found: C, 63·5; H, 6·7. $C_{14}H_{18}O_5$ requires C, 63·1; H, 6·8%). When the vigorous reaction between this ester (20 g.) and sodium (2·5 g.) in benzene (100 c.c.) had ceased, the mixture was heated on the steam-bath for 1·5 hours and next day the sodio-derivative was collected and decomposed with dilute acetic acid. The resulting *carbethoxycoumaranone* separated from light petroleum (b. p. 80—100°) in colourless prisms (13 g.), m. p. 80—81°, which gave a dark green coloration with alcoholic ferric chloride (Found: C, 65·3; H, 5·4. $C_{12}H_{12}O_4$ requires C, 65·5; H, 5·5%). The slow spontaneous transformation of this crystalline coumaranone into a viscous oil is probably due to partial formation of the ketonic form.

7-Hydroxy-7′-methylcoumarono-(2′ : 3′ : 3 : 4)-coumarin (3·1 g.) was obtained by the interaction of the foregoing carbethoxycoumaranone (3·5 g.) and resorcinol (5 g.) in 85% sulphuric acid during 2 days; it separated from warm alcohol as a microcrystalline powder (2·7 g.) which did not melt below 300° (Found: C, 71·8; H, 3·7. $C_{16}H_{10}O_4$ requires C, 72·2; H, 3·8%). The *acetate* formed long slender needles, m. p. 251°, from ethyl acetate (Found: C, 70·4; H, 4·1. $C_{18}H_{12}O_5$ requires C, 70·2; H, 3·9%). Prepared by methylation of the coumarin (0·15 g.) with excess of silver oxide and methyl iodide (2 c.c., added in 5 portions) in boiling ether (20 c.c.) during 8 hours, the *methyl ether* separated from methanol in small needles, m. p. 234° (Found: C, 73·0; H, 4·6. $C_{17}H_{12}O_4$ requires C, 72·9; H, 4·3%).

When resorcinol was replaced by phloroglucinol or pyrogallol in the foregoing condensation,

crystalline products could not be isolated. With these phenols the hydrogen chloride method also gave negative results.

Ethyl 6-Methoxy-β-coumaranone-2-carboxylate.—Condensation of ethyl 4-methoxysalicylate (Gomberg and Johnson, *J. Amer. Chem. Soc.*, 1917, **39**, 1687) (51 g.) with ethyl bromoacetate (75 ml.) in the presence of potassium carbonate (85 g.) in boiling acetone (350 ml.) in the course of 6 hours (until a sample gave a negative ferric reaction) gave *ethyl 5-methoxy-2-carbethoxyphenoxyacetate* (53 g.) as an oil, b. p. 152—155°/0·1 mm. (Found: C, 59·2; H, 6·1. $C_{14}H_{18}O_6$ requires C, 59·6; H, 6·4%). Interaction of this compound (21 g.) with pulverised sodium (3·2 g.) in boiling benzene (150 ml.) during 2 hours gave a solid (27·5 g.) which on decomposition with warm acetic acid (40 ml.) and subsequent addition of water (400 ml.) gave the *carbethoxycoumaranone* (13 g.), forming rectangular plates, m. p. 112°, from alcohol (Found: C, 61·1; H, 5·0. $C_{12}H_{12}O_5$ requires C, 61·0; H, 5·1%). This substance has a green ferric reaction in alcohol.

7-Hydroxy-6′-methoxycoumarono-(2′ : 3′ : 3 : 4)-coumarin was prepared from the foregoing ester and resorcinol by the hydrogen chloride method; on crystallisation from hot nitrobenzene it formed micaceous plates, m. p. > 300°, almost insoluble in the usual organic solvents (Found: C, 68·3; H, 3·7. $C_{16}H_{10}O_5$ requires C, 68·1; H, 3·5%). Methylation of the coumarin by the methyl iodide–potassium carbonate method gave the *methyl ether* which separated from acetic acid or much alcohol in tiny rods, m. p. 224·5—225° (Found: C, 68·9; H, 4·2. $C_{17}H_{12}O_5$ requires C, 68·9; H, 4·1%). The *acetate* crystallised from acetic acid in small needles, m. p. 248—249° (Found: C, 66·5; H, 3·9. $C_{18}H_{12}O_6$ requires C, 66·7; H, 3·7%).

7 : 8-Dihydroxy-6′-methoxycoumarono-(2′ : 3′ : 3 : 4)-coumarin (hydrogen chloride method) separated from nitrobenzene in rod-like prisms, m. p. > 300°, having a pale green ferric reaction in alcohol (Found: C, 63·9; H, 3·5. $C_{16}H_{10}O_6$ requires C, 64·4; H, 3·4%). The *diacetate* formed small needles, m. p. 237—238°, from aqueous acetic acid (Found: C, 62·6; H, 4·0. $C_{20}H_{14}O_8$ requires C, 62·8; H, 3·7%), and the *dimethyl ether* crystallised from acetic acid in tiny prisms, m. p. 237—238° (Found: C, 66·2; H, 4·4. $C_{18}H_{14}O_6$ requires C, 66·3; H, 4·3%). A mixture of the ether and the acetate began to melt at 218°.

5 : 7-Dihydroxy-6′-methoxycoumarono-(2′ : 3′ : 3 : 4)-coumarin (hydrogen chloride method), which could not be satisfactorily purified, gave a *dimethyl ether* forming tiny prisms, m. p. 257—258°, from acetic acid (Found: C, 66·5; H, 4·5. $C_{18}H_{14}O_6$ requires C, 66·3; H, 4·3%).

Attempts to condense ethyl 6-methoxy-β-coumaranone-2-carboxylate with *o*-, *m*-, or *p*-cresol were unsuccessful.

7-Hydroxyindolo-(2′ : 3′ : 3 : 4)-coumarin (III).—The dark-brown solution of methyl 3-hydroxyindole-2-carboxylate (1 g.) and resorcinol (3 g.) in 90% sulphuric acid gradually deposited a yellow solid. After 2 days ice was added, and the yellow precipitate on being isolated and well washed with water became dark green. Crystallised several times from 50% alcohol (charcoal), this product gave the *indolocoumarin* as a colourless microcrystalline solid (1·2 g.) which did not melt below 315° (Found: C, 71·3; H, 3·7; N, 5·4. $C_{15}H_9O_3N$ requires C, 71·7; H, 3·6; N, 5·6%). Acetylation of this compound by the pyridine method gave the *diacetyl* derivative which separated from ethyl acetate in long, slender, colourless needles, m. p. 232° (Found: C, 68·1; H, 4·0. $C_{19}H_{13}O_5N$ requires C, 68·1; H, 3·9%).

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340. *Hydroxy-carbonyl Compounds. Part XIII. Extensions of the Wolff Rearrangement.*

By F. M. DEAN and ALEXANDER ROBERTSON.

Interaction of a series of acid chlorides with diazo-ethane, -*n*-propane and -*n*-butane yields the corresponding diazoketones, (type II, R = alkyl), which have been converted respectively into the amides of the next higher homologues of the parent acids, type (III, R_1 = alkyl). Compared with those in the diazomethane series, the yields obtained in the present work are relatively poor.

FOLLOWING upon the clarification of the course of the reaction between diazomethane and acid chlorides (Nierenstein *et al.*, *J.*, 1915, **107**, 1491; *J. Amer. Chem. Soc.*, 1925, **47**, 1728) by Arndt and his co-workers (*Ber.*, 1927, **60**, 1364; 1928, **61**, 1122) and by Robinson and Bradley (*J.*, 1928, 1310), a convenient method became available for the preparation of diazoketones of the type (II), and consequently it became possible to utilise, as a general synthetical procedure, the rearrangement discovered by Wolff (*Annalen*, 1912, **394**, 25) whereby these diazens (II) can be readily converted into the acids (III) or their amides; conditions for the conversion of (II) into the ester corresponding to (III) were first described by Arndt and Eistert (*Ber.*, 1935, **68**, 200). In recent years this procedure has found wide application in synthetical work but the examples quoted so far have been confined mainly to the use of diazomethane, although one case with diazoethane has been reported (Eistert, *Angew. Chem.*, 1941, **54**, 124) without experimental details and the rearrangement of several ketones (type II, $R_1 = CO_2Et$) derived from esters of diazoacetic acid have been studied (Eistert, *loc. cit.*; Schroeter, *Ber.*, 1916, **49**, 2704).

In the course of a synthetical investigation where it became necessary to prepare α-methyl-substituted aliphatic acids, the application of the Wolff rearrangement to diazoketones from diazoethane appeared to be a convenient route, and in view of the possible value of this kind of extension of the reaction it was decided to examine the behaviour of ketones formed by the action of acid chlorides on diazo-ethane, -*n*-propane, and -*n*-butane. One of the drawbacks to the employment of the Wolff reaction in this way is the relative inaccessibility of the higher diazohydrocarbons, although to a considerable extent the procedure of Adamson and Kenner (*J.*, **1937**, 1551) overcomes this.

$$\underset{\text{(I.)}}{R\cdot CO\cdot Cl} \longrightarrow \underset{\text{(II.)}}{R\cdot CO\cdot CR_1{:}N_2} \longrightarrow \underset{\text{(III.)}}{R\cdot CHR_1\cdot CO_2H}$$

The method employed in the present work for the preparation of diazoketones was essentially the same as for the derivatives of diazomethane, but in the examples studied, with the exception of *p*-nitrobenzoyldiazoethane, the products were obtained as oils, probably owing, in part at least, to the presence of unavoidable by-products of the reaction; *e.g.*, it is impossible to eliminate entirely the formation of chloro-ketone. In the course of attempts to purify the diazoketones it was found, for example, that chromatography of the product from *p*-methoxybenzoyl chloride and diazoethane in benzene on a column of alumina gave a uniform yellow zone, but on elution the diazoketone did not solidify at room temperature whereas the corresponding diazoketone from α-naphthoyl chloride began to decompose on the column. Characterisation of the diazoketones was effected in several cases by the direct interaction of the compound with *p*-nitrobenzoic acid to give the *p*-nitrobenzoate of the ketol (compare Bradley and Schwarzenbach, *J.*, **1928**, 2904). Conversion of the diazoketones into the ketols was carried out in a number of cases by hydrolysis with dilute formic or sulphuric acid but the products were impure, giving several yellow zones when chromatographed on alumina.

The diazoketones of type (II, R_1 = alkyl) have been subjected to the Wolff rearrangement, and because of the difficulty of purifying small amounts of liquid or low-melting acids and their esters, the amide reaction was chosen. In the main the yields of acids or acid derivatives from diazoketones obtained from diazomethane seem to vary between from 40% to 80%, for the cases which have been described, but in the present series, with the exception of phenylbenzoyldiazomethane, the diazoketones of type (II, R_1 = alkyl) gave yields which varied from 10% to 30%, and allowance being made for the presence of impurities in the crude diazoketones it seems certain that in general the yields are decidedly inferior and more resinous by-product is formed, probably owing, in part at least, to the polymerisation of the hypothetical radical $R\cdot CO\cdot C(R_1)$:. As far as can be judged at present the yield from (II) decreases as R_1 is changed in the order Ph > $CO\cdot CH_3$ (or CO_2Et) > H > alkyl.

Experimental.

1-p-*Nitrobenzoyldiazoethane*.—The addition of *p*-nitrobenzoyl chloride (4·5 g.,) dissolved in ether (50 ml.), to an excess of ethereal diazoethane maintained at 0° in the course of 20 minutes, followed ½ hour later by the evaporation of the excess of diazoethane and the solvent in a vacuum, gave the *diazoketone* (4 g.), which formed pale yellow prisms, m. p. 112°, from benzene–light petroleum (b. p. 60—80°), readily soluble in benzene or ethyl acetate and moderately soluble in alcohol and developing an orange coloration with zinc dust and ammonia (Found: N, 20·9. $C_9H_7O_3N_3$ requires N, 20·5%). Attempts to effect the Wolff rearrangement with this diazen were unsuccessful (compare Walker, *J.*, 1940, 1304).

α-p-*Methoxyphenylpropionamide*.—Interaction of anisoyl chloride with an excess of diazoethane in ether gave a liquid diazoketone which did not solidify on being kept and gave a deep purple coloration with zinc dust and ammonia in alcohol. Chromatographic adsorption of this material on a column of alumina with benzene as the solvent furnished a single yellow zone which on elution yielded an oil. A solution of this material (0·5 g.) and *p*-nitrobenzoic acid (0·5 g.) in acetone (20 ml.) was refluxed until a sample did not evolve nitrogen on the addition of a drop of concentrated hydrochloric acid (3 hours). An ethereal solution of the residue obtained on evaporation of the acetone was washed with aqueous sodium hydrogen carbonate to remove acid, dried, and evaporated, leaving a yellow paste which on repeated crystallisation from acetone gave the p-*nitrobenzoate* of *p*-methoxybenzoylmethylcarbinol in pale yellow needles, m. p. 122° (sintering at 118°) (Found: N, 4·4; *M*, 300. $C_{17}H_{15}O_6N$ requires N, 4·3%; *M*, 329).

Decomposition of the crude diazoketone (1 g.) dissolved in acetone (25 ml.) with 20% formic acid (5 ml.) or 2N-sulphuric acid (5 ml.) during 30 minutes gave rise to the ketol as a yellow oil, readily reducing Fehling's solution. Acylation of this product with *p*-nitrobenzoyl chloride and pyridine gave a small yield of the *p*-nitrobenzoate, m. p. and mixed m. p. 121—122°, identical with the ester prepared directly from the diazoketone.

The foregoing diazoketone (1 g.), dissolved in ether (25 ml.), was added to a solution (with shaking) of 10% silver nitrate (2 ml.) in aqueous ammonia (10 ml., *d* 0·880) and water (50 ml.), and the mixture kept at 70° for 2 hours, cooled, and extracted with ether. Evaporation of the dried extract left a yellow

product which partly solidified. Chromatographic adsorption of this material dissolved in benzene on a column of alumina gave several coloured zones with a larger colourless section between the top and the second coloured zone. The colourless section was separated and the ***amide*** (0·26 g.) eluted with alcohol and crystallised from water, forming colourless needles or plates, m. p. 109·5—110° (Found: C, 67·5; H, 7·4; N, 8·0. $C_{10}H_{13}O_2N$ requires C, 67·0; H, 7·3; N, 7·8%).

α-1-*Naphthylpropionamide*.—Prepared the interaction of *α*-naphthoyl chloride and diazoethane in ether, the diazoketone was obtained as a yellow oil which gave a purple-violet coloration with zinc dust and ammonia in alcohol. A solution of this compound (1 g.) in ether (25 ml.) was slowly added to a well-stirred mixture of 10% silver nitrate (2 ml.), ammonia (10 ml., *d* 0·880), and water (50 ml.) kept at 70—80° in an atmosphere of nitrogen for 2 hours. On isolation with ether, the ***amide*** (0·28 g.) was separated from yellow impurities by chromatography (benzene solution) on alumina (elute with alcohol), and crystallised from water, forming colourless prisms, m. p. 129—129·5° ((Found: C, 78·5; H, 6·8. $C_{13}H_{13}ON$ requires C, 78·4; H, 6·6%).

β-*Phenylisobutyramide*.—Prepared by the action of ethereal diazoethane on phenylacetyl chloride the *α*-phenylacetyldiazoethane was an oil, having a deep red zinc dust–ammonia reaction. On being heated with *p*-nitrobenzoic acid (0·5 g.) in a little acetone this substance (0·5 g.) gave the p-*nitrobenzoate* of phenylacetylmethylcarbinol (0·5 g.), forming pale straw-coloured prisms, m. p. 82—83°, from alcohol (charcoal) (Found: C, 65·2; H, 4·8; N, 4·7. $C_{17}H_{15}O_5N$ requires C, 65·2; H, 4·8; N, 4·5%).

Treatment of the diazoketone (0·9 g.) with ammoniacal silver nitrate at 80° for 1 hour gave rise to the *β*-phenyl*iso*butyramide (0·25 g.), which separated from dilute alcohol in elongated plates, m. p. 106—108° (Meyer, *Monatsh.*, 1906, **27**, 1091).

p-Methoxyphenylacetyl chloride and diazoethane gave a liquid diazoketone which, on being heated in acetone with 4-nitro- or 3 : 5-dinitro-benzoic acid gave a resin, and by the ammoniacal silver oxide method gave only a small yield of the *iso*butyramide, m. p. 122—124°, identical with an authentic specimen (Woodruff and Conger, *J. Amer. Chem. Soc.*, 1938, **60**, 466).

γ-(p-*Methoxyphenyl*)-*α*-*methylbutyramide*.—The oil (1 g., having a transient red zinc dust–ammonia reaction) obtained from the interaction of *β*-(*p*-methoxyphenyl)propionyl chloride and diazoethane was refluxed with *p*-nitrobenzoic acid (0·75 g.) in acetone (25 ml.) for 3 hours. On isolation the yellow neutral material partly solidified, and after the removal of an oily contaminant with a little alcohol the residual p-*nitrobenzoate* of *β*-*p*-methoxyphenylpropionylmethylcarbinol formed pale yellow needles (0·45 g.), m. p. 87°, from alcohol, insoluble in ligroin. Decomposition of the diazoketone (1 g.), dissolved in acetone (25 ml.), with aqueous formic acid at 70° for 30 minutes gave the ketol (0·8 g.) which by the pyridine method yielded the same *p*-nitrobenzoate, m. p. and mixed m. p. 87° (Found: C, 63·9; H, 5·5; N, 4·2. $C_{19}H_{19}O_6N$ requires C, 63·9; H, 5·3; N, 3·9%).

When 3 : 5-dinitrobenzoic acid reacted with the diazoketone in acetone a small yield of the corresponding 3 : 5-*dinitrobenzoate* was extracted from the dark viscous product by means of boiling light petroleum (b. p. 60—80°) which separated from chloroform–alcohol in light yellow prisms, m. p. 127·5—128° (Found: N, 6·8. $C_{19}H_{18}O_8N_2$ requires N, 7·0%).

Prepared from the diazoketone by the standard method, *γ*-(p-*methoxyphenyl*)-*α*-*methylbutyramide* formed glistening plates, m. p. 162—163°, from very dilute alcohol (Found: N, 6·5. $C_{12}H_{17}O_2N$ requires N, 6·8.

β-*Phenyl*-*α*-*ethylpropionamide*.—Interaction of phenylacetyl chloride and excess of diazo-*n*-propane (Adamson and Kenner, *loc. cit.*) in ethereal solution yielded a lemon-yellow diazoketone which had a deep red zinc dust–ammonia reaction. This substance (1 g.) was allowed to react with concentrated aqueous ammonia (10 ml.) and 10% silver nitrate solution (2 ml.) at 80° for 2 hours, and on isolation the crude amide (0·85 g.) was purified by chromatographic adsorption from benzene on a column of alumina. Pure material was recovered by means of alcohol from the almost colourless zone between two yellow zones. Crystallised from water, the compound formed colourless needles, m. p. 117° (Found: N, 8·0. Calc. for $C_{11}H_{15}ON$: N, 7·9%). Blicke and Cantolella (*J. Amer. Chem. Soc.*, 1938, **60**, 2926) gave m. p. 117—118°.

Prepared from *β*-*p*-methoxyphenylpropionyl chloride and diazo-*n*-propane, the liquid diazoketone (1 g.) gave under similar conditions a highly impure amide (0·72 g.) which did not solidify and was purified by chromatographic adsorption on alumina from benzene. On isolation *γ*-(p-*methoxyphenyl*)-*α*-*ethylbutyramide* crystallised from water in slender needles, m. p. 105—106° (Found: C, 70·3; H, 8·5; N, 6·7. $C_{13}H_{19}O_2N$ requires C, 70·6; H, 8·6; N, 6·3%).

α-*Benzyl*-n-*valeramide*.—When a solution of phenylacetyl chloride (3 g.) in ether (50 ml.) was slowly added to excess of ethereal diazo-*n*-butane maintained at 0°, and the solvent and butyl chloride were removed in a vacuum one hour later, the diazo-compound remained as an oil having a deep red zinc dust–ammonia reaction. Interaction of this material (0·5 g.) with *p*-nitrobenzoic acid (0·5 g.) in boiling chloroform until a sample did not evolve nitrogen when treated with a drop of concentrated hydrochloric acid gave rise to an oily p-*nitrobenzoate* of the ketol which gradually solidified and then separated from aqueous alcohol in almost colourless needles, m. p. 86° (Found: N, 4·1. $C_{19}H_{19}O_5N$ requires N, 4·1%).

Treatment of the diazoketone dissolved in dioxan with aqueous ammoniacal silver nitrate at 60° during 2 hours gave rise to the *amide*, which crystallised from water in colourless needles, m. p. 121—122° (Found: C, 75·5; H, 9·0; N, 7·3. $C_{12}H_{17}ON$ requires C, 75·4; H, 9·0; N, 7·3%); yield 20%.

An authentic sample of this amide was prepared by the following route: *n*-Propyl bromide was added to a solution of the sodio-derivative of ethyl benzylmalonate (from 3 g. of sodium and 30 g. of ester), and the mixture refluxed until a sample gave a neutral reaction. On isolation, ethyl benzyl-*n*-propylmalonate was purified by distillation in a vacuum and obtained in comparatively large prisms, m. p. 50—52°, b. p. 232°/118 mm. in 87% yield. When this ester (5 g.) was heated with urea (1·5 g.) and alcoholic sodium ethoxide (from 1·2 g. of sodium and 25 ml. of alcohol) in a sealed tube at 130° for 2 hours, the alcohol evaporated, and the residue acidified with concentrated hydrochloric acid, a product was obtained which was partly soluble in 8% aqueous sodium hydroxide. Crystallisation of the alkali-soluble material from alcohol and then benzene gave 5-benzyl-5-*n*-propylbarbituric acid in needles, m. p. 209—210° (Found: C, 64·4; H, 6·1; N, 10·4. Calc. for $C_{14}H_{16}O_3N_2$: C, 64·6; H, 6·1; N, 10·8%) (compare Dox

and Yoder, *J. Amer. Chem. Soc.*, 1922, **44**, 1141, who gave m. p. 210°). The alkali-insoluble material formed elongated, colourless prisms, m. p. 166°, from aqueous alcohol and then benzene (Found : C, 69·2; H, 7·4; N, 9·2%).

When a mixture of ethyl benzyl-*n*-propylmalonate (20 g.), alcohol (134 ml.), and 2N-aqueous sodium hydroxide (134 ml.) was refluxed for 6 hours an excellent yield of the acid ester was obtained which on decarboxylation in glycerol at 110—140° gave ethyl α-benzylvalerate. This ester was purified by distillation and on hydrolysis with N-aqueous sodium hydroxide gave the parent acid, the chloride of which (prepared by means of thionyl chloride), on treatment with ammonium carbonate in ether, gave the amide, m. p. 121—122°, after purification, identical in every way with the specimen obtained from the diazoketone (Found : C, 75·3; H, 8·9%).

Diphenylacetamide.—Phenylbenzoyldiazomethane (Ritter and Wideman, *J. Amer. Chem. Soc.*, 1929, **51**, 3583) (2·1 g.), dissolved in ether (50 ml.), was gradually added to a mixture of ammonia (20 ml., *d* 0·880), 10% silver nitrate solution (4 ml.), and water (100 ml.) at 80°, and the mixture then kept at this temperature for 2 hours; after 1 hour 10% aqueous silver nitrate (2 ml.) was added. Separated from the cooled dark red reaction mixture by filtration, the solid, on extraction with warm alcohol, gave the main bulk of the amide; a further small quantity was isolated from the liquor by means of ether. Recrystallisation of the combined products (1·8 g.) from alcohol gave diphenylacetamide in large prisms, m. p. 167—168°. Evaporation of the alcoholic residues left a deep yellow intractable resin.

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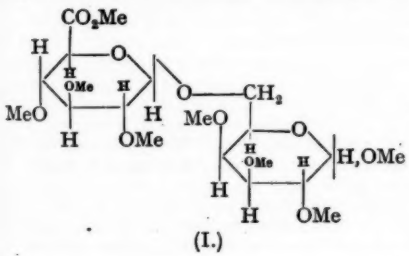
341. *The Structure of Almond-tree Gum. Part I. The Constitution of the Aldobionic Acid derived from the Gum.*

By F. BROWN, E. L. HIRST, and J. K. N. JONES.

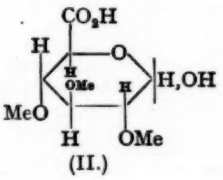
Almond-tree gum on hydrolysis yields L-arabinose (4 parts), D-xylose (2 parts), D-galactose (3 parts), and D-glucuronic acid (1 part). On graded hydrolysis, the polysaccharide yields an aldobionic acid identified as 6-(D-glucuronosido)-D-galactose, since on methylation, followed by hydrolysis of the methylated derivative, 2 : 3 : 4-trimethyl D-glucuronic acid and 2 : 3 : 4-trimethyl D-galactose are produced. The structure of this aldobionic acid is identical with that of the aldobionic acids found in gum arabic and egg-plum gum.

DURING the summer months, the almond tree exudes a gum which is, at first, colourless or pale yellow and very viscid, but gradually hardens, ultimately becoming insoluble in water. In physical properties and appearance it closely resembles those gums which are exuded by other members of the *Rosaceæ* family, *e.g.*, the damson, the cherry, and the egg-plum. Almond-tree gum is the neutral salt of an acidic polysaccharide which can be obtained free from ash on precipitation from acidified aqueous solution by alcohol. When an aqueous solution of the ash-free acidic gum (equiv., 1470) is boiled, hydrolysis occurs with formation of L-arabinose, D-xylose, and a degraded polysaccharide which, on further hydrolysis with 0·1N-sulphuric acid, yields D-galactose and an aldobionic acid. The yield of arabinose (40% of the original weight of the gum) was determined by the paper chromatogram method (Flood, Hirst, and Jones, *Nature*, 1947, **160**, 86). Precipitation of the arabinose as the benzoylhydrazone (Hirst, Jones, and Woods, *J.*, 1947, 1049) gave a result (36%) which, in view of the possible experimental error in the latter method, is in good agreement. D-Xylose (20%) was also detected and determined on the paper chromatogram. Hydrolysis of the degraded polysaccharide yielded D-galactose (23%, calculated on the original weight of the gum) and an aldobionic acid which on further hydrolysis gave D-galactose and D-glucuronic acid in equimolecular proportions.

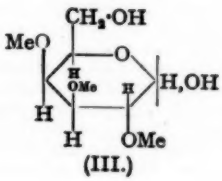
On methylation, the aldobionic acid yielded an octamethyl derivative (I) which gave, on hydrolysis, 2 : 3 : 4-trimethyl D-glucuronic acid (II) and 2 : 3 : 4-trimethyl D-galactose (III). The uronic acid fraction was identified after oxidation and esterification in the form of the crystalline methyl ester of 2 : 3 : 4-trimethyl D-saccharopyranolactone. 2 : 3 : 4-Trimethyl D-galactose was identified as the crystalline sugar and as its crystalline anilide.



(I.)



(II.)



(III.)

The ease of hydrolysis of the arabinose residues indicated that they were combined in the gum in the furanose form. Some evidence was also obtained for the production of an oligosaccharide during the autohydrolysis of the gum, since on boiling with N-sulphuric acid the sugars obtained from the autohydrolysis, the specific rotation of the solution rose from + 31° to + 66°.

It may be significant that the degraded polysaccharide resembled the degraded gums from gum arabic, egg-plum gum, cherry gum, and damson gum, in that it was built up of a repeating unit of one molecule of aldobionic acid combined with two molecules of D-galactose. The aldobionic acid was identical in structure with that isolated from gum arabic and egg-plum gum. Since the yields of aldobionic acid were good it is considered unlikely that any other differently constituted aldobionic acid may be present in the gum.

Experimental.

The gum was collected in Bristol in the summer months of 1939 and 1943 from the branches and trunks of almond trees. It was dissolved in water and filtered, and the filtrate acidified with dilute hydrochloric acid. The solution was poured into methylated spirit and the precipitated material washed with alcohol. Further purification was effected by dissolving the product in cold water and filtering the solution into alcohol. The precipitate was filtered off, washed with alcohol and with ether, and then dried under reduced pressure. The product, a white powder, was easily soluble in water with the formation of an acidic non-reducing solution. The gum did not give an insoluble copper salt [Found: Equiv., 1470 (mean value) by titration with 0·1N-sodium hydroxide; ash 1·0%: $[\alpha]_D^{20°}$ − 44° (*c*, 1·3 as sodium salt in water); galactose residues calc. as $C_6H_{10}O_5$, 33% (from the yield of mucic acid obtained on oxidation of the polysaccharide with nitric acid)].

Autohydrolysis.—A solution of the gum (5·42 g.) in distilled water (100 c.c.) was kept at 98° and the reaction followed polarimetrically: $[\alpha]_D^{20°}$ − 44° (initial value); + 17·7° (23·5 hours); + 23·6° (26·5 hours); + 25·8° (28 hours); + 28·0° (30·5 hours). (A much slower hydrolysis continued beyond this stage.) After 31 hours, barium carbonate was added and the neutral solution filtered. The insoluble barium salts were washed with hot water and the combined filtrate and washings were evaporated under reduced pressure to a syrup which was exhaustively extracted with dry methanol. The soluble fraction (A) (3·23 g.) showed $[\alpha]_D$ + 30·6° (in water). The residue (B) (2·62 g.) was the barium salt of a tetrasaccharide (Found: Ba, 8·7. Calc. for a tetrasaccharide consisting of 3 residues of galactose and 1 residue of glucuronic acid, Ba, 9·1%).

Examination of Soluble Fraction (*A*).—The syrup contained some oligosaccharide since in boiling N-sulphuric acid, its specific rotation increased from $[\alpha]_D$ + 31° to + 66° (constant value) in 30 minutes. The syrup isolated after this further hydrolysis was examined by the paper chromatogram method (Partridge, *Nature*, 1946, **158**, 270) and was found to contain only L-arabinose and D-xylose (by comparison with the results obtained with a synthetic mixture containing these sugars). L-Arabinose was also detected by the formation of L-arabinose benzoylhydrazone [m. p. 186° (decomp.)] and D-xylose by the isolation of its dibenzylidene dimethyl acetal derivative [m. p. 211°; $[\alpha]_D$ − 9° (in chloroform); see Breddy and Jones, *J.*, 1945, 739].

Quantitative determination of L-arabinose by the method of Hirst, Jones, and Woods (*loc. cit.*) indicated the presence of 36% of this sugar in the gum. A determination of arabinose and xylose after separation on the paper chromatogram was carried out by extracting the separated sugar zones with water followed by determination of the sugars with Somogyi's copper reagent (Flood, Hirst, and Jones, *loc. cit.*). The arabinose and xylose were in the molecular ratio 2 : 1 which is in good agreement with the observed equilibrium rotation (+ 66°) of the isolated syrup. A polysaccharide of equiv. 1470 containing 4 residues of arabinose, 2 residues of xylose, 3 residues of galactose, and 1 residue of glucuronic acid should give 42% of arabinose and 21% of xylose on hydrolysis.

Examination of Insoluble Fraction (*B*).—A portion (0·25 g.) was dissolved in 0·1N-sulphuric acid (25 c.c.), the precipitate of barium sulphate removed by filtration, and the filtrate boiled until the rotation became constant: $[\alpha]_D$ + 19·3° (initial value); + 33·5° (1 hour); + 30·5° (2 hours); + 30·6° (5 hours, constant value). From the final figure it follows that the aldobionic acid had a negative rotation ($[\alpha]_D$ *ca.* − 20°). It is therefore a β-D-glucuronosido-derivative. Barium carbonate was added to neutralise the acid, and the sugars were isolated in the usual way. From a portion (0·24 g.) of the resultant syrup, D-galactose (0·11 g., m. p. 167°) was obtained on extraction with methanol. This material gave D-galactose phenylmethylhydrazone (m. p. 179° alone or admixed with an authentic specimen) on treatment with phenylmethylhydrazine. In a quantitative experiment, 0·89 g. of the isolated sugar yielded 1·33 g. of the phenylmethylhydrazone. No sugar other than D-galactose was present. The material (C) (0·13 g.) insoluble in methanol contained 16·4% of Ba (Calc. for $C_{12}H_{19}O_{12}Ba_{\frac{1}{2}}$, Ba, 16·2%).

Methyl Ester of 6-(2 : 3 : 4-*Trimethyl* D-*glucuronosido*) 2 : 3 : 4-*Trimethyl Methyl*-D-*galactoside*.—The barium salt of the aldobionic acid (0·95 g.) (see above) was methylated by dissolving it in water (10 c.c.), and adding methyl sulphate (10 c.c.) and then 30% sodium hydroxide solution (20 c.c.) dropwise in 4 hours with vigorous stirring. After standing overnight the mixture did not reduce Fehling's solution. Solid sodium hydroxide (9 g.) was added followed by methyl sulphate (20 c.c.) dropwise (during 7 hours), and the stirring continued throughout the addition. When all the methyl sulphate had been added, the mixture was heated on the boiling water-bath for 30 minutes. The solution was cooled, acidified with cold concentrated sulphuric acid, and then extracted 5 times with chloroform. Evaporation of the chloroform left a syrup which was again methylated with sodium hydroxide (20 c.c.) and methyl sulphate (10 c.c.). The product was isolated by extracting the acidified solution 5 times with chloroform (see above). The syrup (0·41 g.) obtained on evaporation of the dried chloroform solution was methylated once with silver oxide and methyl iodide. The product (0·41 g.) was then distilled under diminished pressure. No low-boiling fractions were obtained, the absence of monosaccharides being thus indicated,

and the main bulk (0·37 g.) distilled at 170°/0·1 mm. (bath temp.); $n_D^{20°}$ 1·4675 (Found: OMe, 50·4. Calc. for $C_{20}H_{36}O_{12}$: OMe, 52·8%).

Hydrolysis. The methyl heptamethyl aldobionate (0·30 g.) was hydrolysed by boiling it in N-hydrochloric acid (4½ hours). The rotation could not be observed initially, owing to the opalescence of the solution, but after 2½ hours $[\alpha]_D$ was + 52°. Thereafter the solution became too dark to allow of polarimetric observation. The acid was neutralised with silver carbonate and the solution was filtered before and after the passage of hydrogen sulphide. The solution was neutralised with barium carbonate after being aerated to remove hydrogen sulphide. The insoluble barium salts were removed by filtration, and the filtrate evaporated to a syrup (0·26 g.); this was exhaustively extracted with ether. The ethereal extract on concentration gave a syrup (E) (0·11 g.; $n_D^{22·5°}$ 1·4680). The insoluble residue (D) (0·15 g.) consisted of the barium salt of the uronic acid fragment (see below). The syrup (E) crystallised on trituration with a small quantity of ether. It was proved to be 2 : 3 : 4-trimethyl D-galactose by its m. p. (77°) and by its conversion into a crystalline anilide (m. p. 168°, after recrystallisation from absolute ethanol; not depressed on admixture with an authentic specimen of 2 : 3 : 4-trimethyl D-galactose anilide). In cold methanolic hydrogen chloride (1%) the specific rotation rose from + 55° to + 90° in 4 hours and then remained constant. This indicated the absence of a hydroxyl group on C_4 of the galactose derivative.

The uronic acid portion (D) was identified by dissolving the barium salt (0·15 g.) in water and removing the barium as sulphate. After filtration, bromine was added to the clear solution and the oxidation was continued until the solution was non-reducing. The excess of bromine was removed by aeration, and the solution neutralised with silver carbonate. After filtration, hydrogen sulphide was passed into the solution which was again filtered. The filtrate was evaporated under reduced pressure and the residual acid boiled with 1% methanolic hydrogen chloride for 3 hours. The ester was distilled under diminished pressure, giving the methyl ester of 2 : 3 : 4-trimethyl D-saccharopyranolactone, m. p. 110°, not depressed on admixture with an authentic sample.

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342. *Quantitative Analysis of Mixtures of Sugars by the Method of Partition Chromatography. Part I. Standardisation of Procedure.*

By A. E. FLOOD, E. L. HIRST, and J. K. N. JONES.

A method is described for the separation and determination of mixtures of sugars (total weight *ca.* 0·1—1·0 mg.) on the paper chromatogram. The method is accurate to within ± 2%. Polysaccharides in which the ratio of sugars had already been determined have been examined using this new method, and the results are in agreement with those previously recorded.

THE identification and determination of the components of mixtures of sugars have become a matter of great importance in the study of complex polysaccharides such as the plant gums and mucilages. Among the most commonly occurring sugars are galactose, mannose, arabinose, and xylose, to which must be added glucose, fructose, rhamnose, and fucose, which are of less frequent occurrence. Methods for the determination of a single sugar are comparatively simple and have been widely used, but, when mixtures of sugars are to be examined, the problem becomes much more difficult.

Earlier attempts to solve this problem were based on the use of a combination of various analytical methods, the combination being chosen to suit the particular mixture under examination. In favourable circumstances a mixture of two sugars could be determined by a combination of two polarimetric equations, or combined polariscopic and reduction equations (Browne, *J. Amer. Chem. Soc.*, 1906, **28**, 439). Similarly, a mixture of three sugars could be examined by a combination of polarimetric and reduction methods and one selective method, or by two selective methods combined with a determination of total reducing power (Zerban and Wiley, *Ind. Eng. Chem. Anal.*, 1934, **6**, 354). These methods are, by their nature, of limited application.

Attention has been directed to extending the scope of the selective methods. This involves a search for a specific precipitant for each individual sugar. Thus galactose gives an insoluble phenylmethylhydrazone (van Ekenstein and Lobry de Bruyn, *Rec. Trav. chim.*, 1896, **15**, 225), mannose an insoluble phenylhydrazone (Fischer and Hirschberger, *Ber.*, 1888, **21**, 1807), and arabinose an insoluble benzoylhydrazone (Davidis, *Ber.*, 1896, **29**, 2311), although in the last case rhamnose interferes when present in considerable amounts and may, under favourable conditions, be determined as such.

With the above precipitants, the necessity for control experiments has been avoided by carrying out the determination under standard conditions (Hirst, Jones, and Woods, *J.*, 1947, 1048). By a similar technique, xylose can be determined by means of its dibenzylidene-dimethylacetal (Breddy and Jones, *J.*, 1945, 738).

In these methods the specificity of the reagent used is not always complete, *e.g.*, mannose and arabinose interfere with the determination of galactose and have to be removed or themselves determined by some other specific precipitant. The overall accuracy in this type of determination is of the order of 10% on amounts of sugar between 100 to 500 mg.

Another approach to the problem of analysing sugar mixtures is based on selective fermentation methods. It was found possible to utilise differential fermentation by two strains of yeast, of which one ferments galactose and one does not. Quantities of the order of 25—150 mg. of galactose have been determined in this way, the recovery of sugar being 92—98% (Wise and Appling, *Ind. Eng. Chem. Anal.*, 1944, **16**, 28). Similarly, xylose can be fermented with another type of yeast and from 12 to 50 mg. determined, the recovery of sugar being 96—104%. Arabinose, rhamnose, fucose, and glucuronic acid are not fermented (*idem*, *ibid.*, 1945, **17**, 182).

An important advance on the qualitative side was made by Partridge (*Nature*, 1946, **158**, 270), who developed an application of the paper chromatogram (Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224) to the separation of individual sugars. By this technique it was possible to separate completely the components of a sugar mixture. Moreover, it was necessary to use only minute amounts of material. The present paper describes an elaboration of this technique to the determination of a sugar mixture using less than 1 mg. of material. Since the components of the mixture are completely and readily separated, a single reagent, provided it will deal with micro-quantities, serves for the determination of all the components. Such a reagent has been reported by Somogyi (*J. Biol. Chem.*, 1945, **160**, 61), and deals with amounts as small as 0·01 mg. of sugar. Quantities of about 0·1 mg. or more of each component sugar can be built up on the paper by the technique described below and subsequently analysed by the use of this reagent. The accuracy has been previously reported as $\pm$ 5% (Flood, Hirst, and Jones, *Nature*, 1947, **160**, 86 *) but with greater experience it has been found possible to work to accuracies of $\pm$ 2%.

The application of this method to the analysis of synthetic mixtures and the mixture of sugars produced on hydrolysis of polysaccharides is described below. The absolute weights of individual sugars present in the synthetic mixtures have been determined by weighing in, before analysis, a foreign sugar having an R_F value different from the sugars to be determined. This foreign sugar serves as a reference compound, and from a knowledge of the ratio of sugars present the absolute weight of each compound is readily calculated.

The polysaccharides chosen had been analysed previously by other methods. The hydrolysis of these polysaccharides was repeated on the macro-scale and small samples taken for analysis on the paper chromatogram. The results are collected in the Table.

Polysaccharide.	Mol. ratio of sugars present in the products of hydrolysis.					
	Found on the micro-scale.			Previously reported.		
	Arabinose.	Xylose.	Galactose.	L-Arabinose.	D-Xylose.	D-Galactose.
Almond-tree gum [1] ...	2·07	1·0	—	2	1	—
Cherry-tree gum [2] ...	3·7	—	1·0	3·7	—	1
Cholla gum [3]	3·0	0·9	1·0 †	3	1	1 †

[1] Brown, Hirst, and Jones (previous paper).
[2] Jones, *J.*, 1939, 562.
[3] Brown, Hirst, and Jones (forthcoming publication).
† This figure does not include the galactose component of the aldobionic acid fraction. The molecular ratios quoted by Flood, Hirst, and Jones (*Nature*, *loc. cit.*) include this galactose residue.

Up to the present, attempts to resolve DL-mixtures of sugars by the use of an optically active alcohol such as menthol or amyl alcohol in place of butanol have been unsuccessful (see experimental section).

Experimental.

Apparatus.—The apparatus used was similar to that employed by Consden, Gordon, and Martin (*loc. cit.*), but in place of a drain-pipe a bell jar with a ground glass flange at the base was used. The jar measured approximately 55 × 20 cm. (diameter) and rested on a glass plate. The glass trough was supported on 4 glass legs, and a beaker served as a receptacle for the aqueous phase. The filter paper used was Whatman No. 1 and was cut to lengths of 60 cm. The width varied from 9 to 12 cm. according

* An alternative method for the adaption of Partridge's qualitative procedure to quantitative ends has been described by Hawthorne (*Nature*, 1947, **160**, 714), whose work was carried out simultaneously with ours but quite independently, the workers in the two laboratories being unaware of each others' experiments.

to the length of trough being used. The solvents and procedure used were as described by Partridge (*loc. cit.*).

Reagents.—The copper reagent was prepared as described by Somogyi (*loc. cit.*) and stored at 20—25°. The ferricyanide reagent was prepared as described by Hagedorn and Jensen (*Biochem. Z.*, 1923, **135**, 46); in addition a variation of this reagent was used having a buffer of potassium carbonate–potassium hydrogen carbonate (Hawkins, *J. Biol. Chem.*, 1929, **84**, 80).

Procedure.—The solution containing the sugars must be neutral, and the concentration of inorganic ions must be kept to a minimum in order to avoid water-logging of the paper during the subsequent chromatography (cf. Consden, Gordon, and Martin, *loc. cit.*, p. 229). The concentration is adjusted so that it is of the order of 1% with respect to the individual sugars.

A preliminary trial run is useful in indicating the concentration required for the analysis of a particular mixture. It has been found generally convenient to hydrolyse about 10 mg. of a polysaccharide with approximately 0·5 ml. of N-sulphuric acid in small sealed tubes (10 × 0·4 cm.) immersed in a boiling water-bath for the requisite time. On completion of the hydrolysis the tube is cooled and the solution centrifuged. One end of the tube is made slightly enlarged for the purpose of collecting precipitated solids (*e.g.*, barium sulphate). The tube is then opened and the acid neutralised with barium carbonate. It is advisable to add this material cautiously on the end of a very fine glass rod, the end of which is bent at right angles. The rod also serves as a stirrer. If these precautions are not taken there is a danger of excessive frothing and material may be lost. After neutralisation is complete (Congo-red) the solution is again centrifuged and the clear supernatant neutral liquid is ready for analysis.

Two pieces of filter paper are cut to suitable dimensions as described earlier, and spots of the solution are placed about 5 mm. apart along the starting line with a fine glass capillary. The spots are of the order of 0·002—0·004 ml. and must be of uniform size. They must not coalesce, and the two end spots must not be too near the edge of the paper. It is desirable that one of the end spots should be well separated from the next so that subsequently a small strip containing it can be cut from the paper sheet. The place at which this cut is to be made is indicated by a small pencil mark. In this way two paper sheets are prepared and hung on either side of the trough in the bell jar.

The chromatogram is allowed to run for about 45 hours, after which the sheets are removed and dried for 1 hour in an oven at 100°. The sheets are best handled by attaching clips to each end.

After drying, a small strip is cut from each paper sheet at the place marked and thus contains one end spot. The strip is sprayed with ammoniacal silver nitrate solution and returned to the oven for development.

A normal chromatogram having been obtained on the small strip, the position of the sugars can be located and the degree of separation observed. By matching this strip alongside the major sheet from which it was cut, areas can be cut from the latter each containing one only of the separated sugar components. This device has been justified by experiments which have shown (by spraying the whole sheet) that the separated sugars from each spot do lie on parallel horizontal lines providing that the sheet has hung vertically in the chromatographic apparatus. Finally, areas which are free from sugar and equal in size to each of the sugar-containing areas are cut from the same major sheet to serve as paper blanks. The filter paper was not specially purified before use because during the experiment it is completely washed by the solvent (cf. Consden *et al.*, *loc. cit.*, p. 226) and the blanks were always low and very consistent (see later).

The sugars having been separated, the next stage is their quantitative removal from the paper. Two methods are available. One is based on capillary siphoning and has also been used by Dent (*Biochem. J.*, 1947, **41**, 240). This method has been examined in the present work, but the following procedure is preferable since aseptic conditions can be maintained. To the lower coil of a ground glass spiral condenser is hooked a fine glass rod (diameter 1 mm.) having also a small hook at the lower end. The rod is of such a length that it projects below the end of the condenser so that the paper strip can be conveniently attached to it. A boiling tube having a ground-glass joint is attached to the lower joint of the condenser. This is marked so that it is known when it contains 5 ml. of liquid. When the apparatus is assembled, water is introduced through the top of the condenser until the boiling tube contains water to the mark. In this way allowance can be made for water hold-up in the paper strip. Provided the apparatus is completely vertical with the glass rod hanging vertically from the centre of the lower coil of the condenser and the paper hanging vertically and free within the tube, water flows down the condenser spiral, collects in a drop on the lower coil, slides down the glass rod, flushes the paper strip, and drops off the bottom into the boiling tube. This happens also during the subsequent refluxing of the water in the apparatus. The entire apparatus must be free from grease. Two extractors are used side by side, one for extracting the strip of paper containing the sugar and the other for its corresponding paper blank. The time of refluxing was fixed at 30 minutes to allow a wide margin of safety. After 30 minutes no sugar can be detected on the paper strip (ammoniacal silver nitrate).

Two 5 ml. volumes of solution are thus obtained, one serving as a blank and the other containing the sugar. They are already contained in two boiling tubes, and no further transfer of liquid is necessary during the final analysis. To complete this with the copper reagent, two further similar boiling tubes having ground glass joints are taken, and 5 ml. of water introduced into one to serve as a blank, whilst to the other is added 5 ml. of a standard aqueous solution of the sugar that is under analysis. This standard solution is made from pure recrystallised sugar that has been dried in a vacuum to constant weight. Its concentration is such that 5 ml. will contain as nearly as possible the same quantity of sugar as is present in the solution obtained from extraction of the paper strip. A little experience will indicate the required concentration, and it is soon found possible to judge this from the density of the spots on the small sprayed reference strip. In general, quantities of 20—50 mg. per l. are found suitable.

To all 4 tubes are now added 5 ml. of the copper reagent. The contents are gently mixed, and the tubes covered with small glass funnels and placed in a briskly boiling water-bath. Clamps are used for this purpose since the tubes must not be disturbed until after final acidification. After 25 minutes the hot water is sucked out of the bath with a filter pump, cold water being added at the same time until the bath temperature reaches 35°. This temperature is maintained for 10 minutes. Potassium

iodide solution (2·5%; 0·5 ml. when the copper reagent contains 5 ml. of N-potassium iodate per l.) is then carefully run down the side of each tube followed by 1·5 ml. of 2N-sulphuric acid. The latter is squirted into the tube from a small glass syringe so that the contents are instantly mixed and acidified. After acidification each tube is immediately closed by a ground glass stopper and the contents chilled in cold water. The contents of each tube are then titrated with 0·005N-sodium thiosulphate. Phenol-red (1 drop) is added to improve the end point, and a micro-burette graduated directly to 0·01 ml. is used. Just before the end point 2 drops of starch solution (1%) are added. The tubes must be kept stoppered before titration, otherwise serious discrepancies arise owing to loss of iodine by volatilisation.

Four titration figures are thus obtained :

Copper reagent	+ 5 ml. of water = a
do.	+ 5 ml. of standard sugar solution = b
do.	+ 5 ml. of extract from paper blank = c
do.	+ 5 ml. of extract from paper containing sugar = d

$(a - b)$ is equivalent to the amount of copper reduced by 5 ml. of standard sugar solution, and $(c - d)$ is equivalent to the amount of copper reduced by the extract from the paper containing the sugar. If 5 ml. of standard sugar solution contains x mg. of sugar, the weight of sugar in the 5 ml. extract can be calculated by simple proportion and is $x(c - d)/(a - b)$. The "blank" due to the paper is measured by $(a - c)$, and is of the order of 0·1 ml. of 0·005N-sodium thiosulphate. This figure is quite constant and varies little from paper to paper, *e.g.*: paper (1), 0·12, 0·12, 0·10 ml.; paper (2), 0·16, 0·16 ml.; paper (3), 0·09, 0·10 ml.; paper (4), 0·14, 0·11, 0·11 ml.

The technique described ensures that both standard and sample are analysed under identical conditions and that the analysis is based solely on a sugar weight. No other reagent used need be standardised.

The analysis can also be carried out using calibration curves for each sugar. In this case the concentration of the sodium thiosulphate must be known.

After completion of the analysis for the individual sugars, a corresponding weight for each is obtained. If it is certain that the sugars analysed are the sole constituents of the mixture under examination, these weights can be converted directly into molecular ratios. If not, the following procedure is adopted. A foreign sugar known not to be present in the mixture under analysis and having a R_F value different from those of the sugars present is weighed in before chromatography to serve as a reference compound. From a knowledge of all the weight ratios the percentage of any component can then be readily calculated. Such a device is very useful when studying the hydrolysis of a polysaccharide. In this case the reference sugar is best weighed in immediately after hydrolysis and before neutralisation. Thus the reference sugar is involved in the same manipulative processes as the other sugars present.

Instead of the copper reagent, potassium ferricyanide can be used, with the same technique. It is well known (cf. Somogyi, *loc. cit.*, p. 61) that alkaline potassium ferricyanide is not so selective for sugars as is copper, and in this work it was found to be very sensitive to slight traces of grease. In order to obtain satisfactory results it is necessary to wash all apparatus before use with a solution of alkaline ferricyanide followed by much distilled water. On this account the papers must be handled with grease-free forceps and not with the fingers. For these reasons the copper reagent is to be preferred as being more convenient.

In the following analyses all chromatograms were run for 45 hours at 20° using *n*-butanol (40%)–ethanol (10%)–water (50%). Ammonia was added to the aqueous phase to a concentration of 1%.

Analysis of Mixtures of Reducing Sugars.—(a) *Determination of galactose, with arabinose as the reference compound.* Arabinose (10·41 mg.) was weighed into a solution containing galactose (9·58 mg.). The solution was separated on the chromatogram as an approximately 2% solution (total solids). Found by titration : galactose, 0·485 mg.; arabinose, 0·532 mg. The absolute amount of galactose in the solution is therefore 10·41 × 0·485/0·532 mg. = 9·49 mg. (99% recovery).

(b) *Analysis of a mixture of rhamnose and arabinose, with glucose as the reference compound.* Glucose (4·38 mg.) was weighed in to a solution containing rhamnose (9·60 mg.) and arabinose (5·80 mg.). The solution was separated on the chromatogram as an approximately 2% solution (total solids). Found by titration : glucose, 0·124 mg.; rhamnose, 0·261 mg.; arabinose, 0·157 mg. The absolute amounts of rhamnose and arabinose present in the original mixture are therefore, rhamnose, 4·38 × 0·261/0·124 mg. = 9·22 mg. (96% recovery); arabinose, 4·38 × 0·157/0·124 = 5·54 mg. (95·5% recovery).

(c) *Analysis of a mixture of arabinose, xylose, and rhamnose, with galactose as a reference sugar.* Galactose (210 mg.) was weighed into a solution containing arabinose (144 mg.), xylose (117 mg.), and rhamnose (200 mg.). The solution was separated on the chromatogram as an approximately 3% solution. Found by titration : galactose, 0·366 mg.; arabinose, 0·257 mg.; xylose, 0·207 mg.; rhamnose, 0·343 mg. Thus the absolute weights of sugar present in the solution are arabinose, 147 mg. (102% recovery); xylose, 119 mg. (102% recovery); rhamnose, 197 mg. (98·5% recovery).

Determination of Sugars Derived by Hydrolysis of Polysaccharides. Determination of the Molecular Ratios of the Sugars Produced.—(a) *Almond-tree gum.* The gum (5·42 g.) was kept at 98° with water (100 ml.) for 31 hours. The solution was neutralised with barium carbonate and filtered. The filtrate was concentrated under reduced pressure to a syrup which was exhaustively extracted with hot methanol. The extracts were concentrated to a syrup under reduced pressure. A portion of the syrup (3·23 g.) was hydrolysed with N-sulphuric acid (25 ml.) for 30 minutes in order to hydrolyse any oligosaccharides. The solution was neutralised with barium carbonate, filtered, and evaporated to a syrup as above. A portion of the syrup (20 mg.) was dissolved in water (1 ml.), and a small portion of it separated on the chromatogram. It was found that arabinose and xylose were present. In a quantitative experiment the portions of paper containing these sugars were found to contain respectively 0·224 mg. of arabinose and 0·108 mg. of xylose. These sugars were present therefore in the molecular ratio 2·07 : 1. Material other than free sugar was detected on the chromatogram, and from its position was either unhydrolysed material, or a uronic acid fraction, or both.

Estimation on the macro-scale indicated that the syrup contained L-arabinose (2 parts) and D-xylose (1 part) (Brown, Hirst, and Jones, *loc. cit.*).

(b) *Cherry-tree gum.* The gum (3·71 g.) was heated at 100° with N-sulphuric acid (100 ml.) for 6·25 hours, by which time the rotation had become constant. The solution was neutralised with barium carbonate, and the syrup isolated as described above. The syrup was dissolved in water, and a portion separated on the chromatogram as an approximately 2% solution (total solids). It was found that arabinose and galactose were present. In a quantitative experiment the portions of paper containing these sugars were found to contain respectively 0·763 mg. of arabinose and 0·251 mg. of galactose. These sugars were present therefore in the molecular ratio 3·7 : 1. Earlier work (Jones, *loc. cit.*) had indicated that the molecular ratio of arabinose to galactose in the hydrolysis products of the gum was 3·7 : 1.

(c) *Cholla gum.* The gum (64 mg.) was hydrolysed by boiling with N-sulphuric acid (10 ml.) for 90 minutes, by which time the rotation had become constant. The solution was neutralised with barium carbonate and the syrup isolated as before. A small portion of the syrup in water was separated on the chromatogram as an approximately 2·5% solution (total solids). Arabinose, xylose, and galactose were found to be present. In a quantitative experiment the portions of paper containing these sugars were found to contain 0·271 mg. of arabinose, 0·081 mg. of xylose, and 0·108 mg. of galactose. These sugars were present therefore in the molecular ratio 3 : 0·9 : 1.

Examination of the gum by macro-methods (Brown, Hirst, and Jones, *loc. cit.*) indicated that the hydrolysis products included L-arabinose (3 parts), D-xylose (1 part), and D-galactose (1 part), in good agreement with the chromatogram results.

Attempted Resolution of DL-*Arabinose* (with W. G. C. Forsyth).—An attempt was made to resolve DL-arabinose (1% aqueous solution) with (*a*) *lævo*menthol, and (*b*) *lævo*amyl alcohol, in place of butanol as the organic phase in the chromatogram. After 160 hours at 20° no separation of the sugars in the racemic mixture was observed.

The University, Manchester. [*Received, December 4th*, 1947.]

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343. *Addition of Thiol Compounds to the Double Bond. Part I. Preparation of Tertiary Mercapto-acids.*

By Zoltan Földi and Janos Kollonitsch.

Addition of hydrogen sulphide and of benzylthiol to *iso*propylidene compounds has been studied. Several β-mercapto-acids and derivatives containing the tertiary β-mercapto-group have been prepared.

The main object of the present work was to prepare β-mercapto*iso*valeric acid and derivatives. Our first attempts, which started from ethyl β-bromo*iso*valerate or from β-bromo*iso*valeric acid, were unsuccessful. Replacement of the bromine atom by the thiol group or substitututed thiol group did not occur, as in each case hydrogen bromide was split off, giving rise to ethyl ββ-dimethylacrylate or ββ-dimethylacrylic acid. For this reason, our interest turned to addition of hydrogen sulphide and of thiols to unsaturated acids.

Addition of hydrogen sulphide, of thiophenols, and of mercapto-acids to αβ-unsaturated acids and ketones has been the subject of numerous investigations (Wallach, *Annalen*, 1894, **279**, 385; 1905, **343**, 32; Posner, *Ber.*, 1901, **34**, 1395; 1902, **35**, 799; 1904, **37**, 502; 1905, **38**, 646; 1907, **40**, 4788; Axberg and Holmberg, *Ber.*, 1933, **66**, 1194; Holmberg, *Brit. Abstr.*, 1944, *A*, II, 2943; Nicolet, *J. Amer. Chem. Soc.*, 1935, **57**, 1098; Carter *et al.*, *J. Biol. Chem.*, 1941, **139**, 247).

In exploratory experiments we prepared methyl 2-mercapto*iso*butyl ketone by the action of liquid hydrogen sulphide on mesityl oxide, in order to collect some information on the efficiency of various catalysts. Triethylamine proved to be an excellent catalyst, affording yields up to 80% of the theory, while diethylamine led to yields of only about 10%. Under similar conditions ethyl ββ-dimethylacrylate showed no appreciable tendency to add either hydrogen sulphide or ethylthiol. Free ββ-dimethylacrylic acid proved to be still more resistant to addition, though a large excess of triethylamine was used. As the vicinity of only one carboxyl group to the double bond proved to be quite inadequate to induce addition, we investigated the addition to ethyl *iso*propylidenemalonate. By using considerably larger amounts of triethylamine than used with mesityl oxide, we obtained *ethyl β-mercapto-α-carbethoxyisovalerate* in yields up to 60% of theory. Attempts, however, to hydrolyse this ester to the corresponding acid were unsuccessful. Alkaline hydrolyses in aqueous or alcoholic solution effects a rather complex disintegration of the molecule, such as splitting off of considerable amounts of hydrogen sulphide, affording *iso*propylidenemalonic acid, and cleavage to malonic acid. Acid hydrolysis was also unsatisfactory.

The synthesis of β-*mercapto*iso*valeric acid* was finally achieved by reaction of hydrogen

sulphide with *iso*propylidenemalonic acid. Under mild conditions, β-mercapto-α-carboxy-*iso*valeric acid was formed in satisfactory yield; on decarboxylation by heating at about 150°, this gave the desired β-mercapto*iso*valeric acid. That the thiol group is a tertiary one is shown by the green coloration in the Rheinboldt test (*Ber.*, 1927, **60**, 184). The addition is therefore " normal " with respect to Markownikoff's rule.

We have also obtained under similar conditions from *iso*propylidenemalonic acid and benzylthiol β-*benzylthio*-α-*carboxy*isovaleric acid, and from ethyl α-nitro-ββ-dimethylacrylate (Bouveault and Wahl, *Compt. rend.*, 1900, **131**, 687, 748) and benzylthiol *ethyl* α-*nitro*-β-*benzylthio*isovalerate. (The constitution of the latter is in doubt, for the compound is insoluble in aqueous sodium hydroxide.)

These reactions show a much greater reactivity of the double bonds in these compounds than in ββ-dimethylacrylic acid; this is in accordance with observations of Scheiber and Meisel (*Ber.*, 1915, **48**, 248, 264), who investigated the reactions of ethyl acetoacetate with ethyl ββ-dimethylacrylate and with ethyl *iso*propylidenemalonate and found the latter, likewise, more reactive.

Experimental.

*Methyl β-Mercapto*isobutyl *Ketone*.—Into a mixture of mesityl oxide (35·5 g.), triethylamine (3 g.), and dry ether, cooled by an acetone–carbon dioxide freezing mixture, dry hydrogen sulphide is introduced until an increase by volume of about 10—15 c.c. takes place. The mixture is then kept in an autoclave at room temperature overnight and finally heated at 50° for 2 hours. The mixture yields, on distillation under 15 mm., after a little forerun, 38 g. of the mercapto-ketone (b. p. 56—60°) as a colourless liquid with a pungent mercaptan odour, and giving the Rheinboldt test characteristic of a tertiary thiol group. For characterisation, the *mercury mercaptide* has been prepared. To the mercapto-ketone (1·32 g.) saturated aqueous mercuric chloride solution (22 c.c.) is added. A voluminous, white precipitate occurs which, after standing overnight, is collected, washed with water, and dried in a desiccator over sulphuric acid; yield, 2·25 g., m. p. 73—75° (not sharp) [Found : Hg, 42·2. $(C_6H_{11}OS)_2Hg$ requires Hg, 43·2%].

Mesityl oxide (20·5 g.) and diethylamine (1 c.c.), treated as above with hydrogen sulphide, yielded only 3 g. of the mercapto-ketone, after a large forerun of mesityl oxide.

*Ethyl β-Mercapto-α-carbethoxy*isovalerate.—A mixture of ethyl *iso*propylidenemalonate (50 g.), triethylamine (30 g.), and absolute ethanol (30 c.c.) was saturated with hydrogen sulphide as described above and then kept in a steel bomb at room temperature for 48 hours and warmed for 2 hours in a water-bath at 70°. The reaction mixture, a colourless liquid mixed with crystals, was diluted with water (30 c.c.), acidified with 5N-hydrochloric acid (80 c.c.), and extracted with ether; the ethereal layer was dried (Na_2SO_4), freed from ether, and the residue (52 g.) fractionated. After a slight forerun, 35 g. of *ethyl β-mercapto-α-carbethoxy*isovalerate were obtained as a colourless liquid, b. p. 90—93°/1 mm. (Found : S, 13·3. $C_{10}H_{18}O_4S$ requires S, 13·6%). The substance has a characteristic mercaptan-like odour. Its solution in ether gives with sodium nitrite and sulphuric acid a green coloration (tertiary mercapto-group). It gives with aqueous mercuric chloride a white precipitate, reduces Fehling's solution, and gives an intense carmine-violet coloration with sodium nitroprusside.

Alkaline hydrolysis. The ester (2·34 g.) is heated with 2·5N-sodium hydroxide (10 c.c.) for 1½ hours on the steam cone, then cooled and acidified with concentrated hydrochloric acid (3 c.c.). Hydrogen sulphide is evolved and an oil separates, which is taken up in ether. The residue (0·96 g.) from the ethereal layer partly crystallises on standing, and after being washed with ethyl chloride affords 0·2 g. of white crystals, m. p. *ca.* 105° (indef.) (equiv., *ca.* 76). It is soluble in water, reduces Fehling's solution, and gives a precipitate with aqueous mercuric chloride. The substance is believed to be the β-lactone of β-mercapto-α-carboxy*iso*valeric acid. The residue from the ethyl chloride mother-liquor, dissolved in water, yields on addition of ammonia and calcium chloride and on standing, a white precipitate of *calcium β-mercapto-α-carboxy*isovalerate (0·07 g.) (Found : Ca, 15·5; H_2O, by drying at 110°, 17·2. $C_6H_8O_4SCa,2\frac{1}{2}H_2O$ requires Ca, 15·4; H_2O, 17·3%).

The aqueous layer from which the separated oil has been removed by ether is further extracted with ether. The ethereal extract yields, on addition of ammoniacal calcium chloride and on standing, white sandy crystals of *calcium iso*propylidenemalonate (0·32 g.) (Found : Ca, 15·5; H_2O, by drying at 110°, 30·1. $C_6H_6O_4Ca,4\frac{1}{2}H_2O$ requires Ca, 15·2; H_2O, 30·8%).

The aqueous solution which has been extracted with ether is evaporated to dryness under diminished pressure, and the solid residue thoroughly extracted with ether. On evaporation of the ether, white leaflets (0·44 g.) are obtained which yield, on addition of ammoniacal calcium chloride, white sandy crystals of calcium malonate (0·65 g.) (Found : Ca, 21·8; H_2O, 22·8. Calc. for $C_3H_2O_4Ca,2\frac{1}{2}H_2O$: Ca, 21·4; H_2O, 24·1%).

*β-Mercapto-α-carboxy*isovaleric *Acid*.—*iso*Propylidenemalonic acid (11·2 g.), dissolved in alcohol (15 c.c.), was mixed with triethylamine (25 c.c.) under cooling with ice, and saturated with hydrogen sulphide while cooled in solid carbon dioxide–acetone. Then the mixture was kept in a steel bomb overnight at room temperature and finally warmed for 2 hours at about 50°. The contents were diluted with ether and acidified with hydrochloric acid. The ethereal layer was dried (Na_2SO_4) and gave on evaporation of the solvent a white crystalline residue. This was extracted with hot chloroform; the mercapto-acid was precipitated from the chloroform solution with light petroleum (b. p. 55—60°). About 10 g. of the crude *mercapto-acid* were obtained, m. p. 115—125° (decomp.). It was purified through two more precipitations with light petroleum from chloroform solution. The pure acid forms long colourless needles, m. p. 136—137° (decomp.) (Found : S, 18·3. $C_6H_{10}O_4S$ requires S, 18·0%), and gives a green coloration in the Rheinboldt test, and a cobalt-blue coloration with aqueous ferric chloride solution.

β-Merçaptoisovaleric Acid.—The foregoing acid (7·9 g.) was decarboxylated by heating at 140—150° for ½ hour. The loss of weight was 2·08 g. (Calc.: 1·98 g.). The remaining oil was distilled at 1 mm. pressure; the fraction, b. p. 74—80°, is a colourless liquid with the odour of both a mercaptan and *iso*valeric acid; it solidified on standing to a white crystalline mass, m. p. *ca.* 20—23°. On refractionation, the fraction of b. p. 112—115°/10 mm. solidified in the receiver and had m. p. 34—35°. (Found: SH, by iodometric titration, 24·6; equiv., 133. $C_5H_{10}O_2S$ requires SH, 24·6%; equiv., 134). The *mercapto-acid* is sparingly soluble in water, and gives with ferric chloride a cobalt-blue coloration.

β-Benzylthio-α-carboxyisovaleric Acid.—*iso*Propylidenemalonic acid (7·2 g.) was dissolved in 85% alcohol (30 c.c.) and mixed under ice-cooling with diethylamine (10 c.c.), benzylthiol (8 c.c.), and triethylamine (5 c.c.); a clear solution was obtained. The flask was hermetically closed, then warmed for 5 mins. in a steam-bath and kept overnight at room temperature. The solvents and the excess of diethylamine and triethylamine were evaporated in a vacuum and the residue was mixed with water (60 c.c.) and 5N-hydrochloric acid (30 c.c.); 12 g. of crude *β-benzylthio-α-carboxyisovaleric acid* separated, m. p. 122—126°. The crude acid was dissolved in ether (25 c.c.) and precipited with light petroleum (70 c.c.), giving 10 g. of the pure acid, m. p. 133—134° (decomp.) (Found: equiv., 273. $C_{15}H_{16}O_4S$ requires equiv., 268).

Ethyl α-Nitro-β-benzylthioisovalerate.—Ethyl α-nitro-ββ-dimethylacrylate (1·73 g.) was mixed with anhydrous ethanol (3 c.c.), and triethylamine (0·1 c.c.) and benzylthiol (1·34 g.) were added. A clear reddish solution was obtained; the temperature of the mixture rose to 60° and it became thicker. The flask was closed and kept overnight. The reaction mixture was then dissolved in ether (15 c.c.), the ethereal layer washed with N-hydrochloric acid (2 × 10 c.c.) and then with water (2 × 5 c.c.), dried (Na_2SO_4), and freed from ether, and the residue kept under 0·3 mm. at 120°. The resulting oil (2·6 g.) was distilled: b. p. 160—162°/0·3 mm.; the *ester* is insoluble in N-sodium hydroxide (Found: S, 10·0. $C_{14}H_{19}O_4NS$ requires S, 10·7%).

The authors' thanks are due to the staff of the Analytical Department of Chinoin Works for the analyses.

RESEARCH LABORATORIES OF CHINOIN WORKS,
UJPEST, HUNGARY. [*Received, November 4th*, 1947.]

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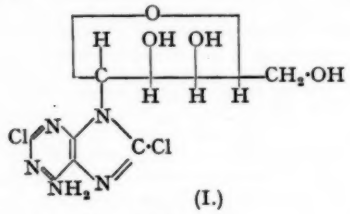
344. *Experiments on the Synthesis of Purine Nucleosides. Part XX. A Synthesis of Guanosine.*

By J. DAVOLL, B. LYTHGOE, and A. R. TODD.

2 : 8-Dichloro-9-β-D-ribofuranosidoadenine (Davoll, Lythgoe, and Todd, this vol., p. 967) has been converted into 9-β-D-*ribofuranosidoguanine*, identical with the natural nucleoside, guanosine. The structures accepted for guanosine and xanthosine are thereby confirmed, and they are shown to possess a β-type of glycosidic linkage.

IN Part XIX (this vol., p. 967) we described a synthesis of adenosine starting from 2 : 8-dichloro-9-β-D-ribofuranosidoadenine (I). Reactions designed to convert glycosides of 2 : 8-dichloroadenine into those of guanine had already been explored by Fischer and Helferich (*Ber.*, 1914, **47**, 210), so that the dichloro-compound (I) was clearly of potential value as a starting point for a synthesis of guanosine (II). Our experiences in effecting this synthesis are reported in the present paper.



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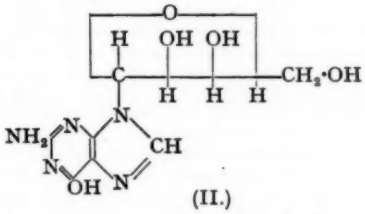

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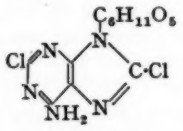

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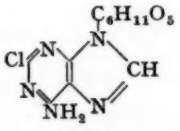
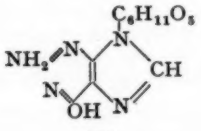


(IV.)



(V.)

Fischer and Helferich's experiments were carried out with "2 : 8-dichloroadenine glucoside" (III) as starting material (for details of the structure of this compound, see Part XIX, *loc. cit.*). Treatment with zinc dust and water replaced one chlorine atom by hydrogen; the

resulting monochloroadenine glucoside was considered tentatively to retain the chlorine atom at position 2 (IV), a view based upon its behaviour in the following reactions. Treatment of (IV) with nitrous acid replaced the amino-group by a hydroxyl group, giving a chlorohypoxanthine glucoside which, without isolation in a state of purity, was heated with ethanolic ammonia to replace the chlorine atom by an amino-group. Owing to lack of material, the final product was not obtained quite pure, but it behaved as a guanine glucoside (V), giving on acidic hydrolysis a substance with the properties of guanine. The difficulties of characterising this purine satisfactorily are well known, and perhaps on this account Fischer and Helferich did not feel able to state with certainty that the reactions followed the course indicated above, and they promised a more detailed investigation at a later date; so far as we are aware, however, this was not carried out.

In order to gain experience with the reactions involved, it was thought desirable first to repeat the above work. No difficulty was experienced in obtaining the final glucoside in an analytically pure condition and with characteristics substantially the same as those originally reported. Its properties satisfied us that it was in all probability a derivative of guanine, and we were able to proceed with the application of similar methods to the dichlororibofuranoside (I). We had found in the course of work with the glucoside (III) that catalytic reduction offered a rapid and convenient alternative to treatment with zinc dust and water as a method for conversion into the monochloro-derivative (IV). The hydrogenation method was therefore applied to (I), and the product of reduction deaminated directly to the corresponding monochlorohypoxanthine glycoside, which, after a preliminary purification through the lead salt, was aminated by heating it with ethanolic ammonia. The crystalline 9-β-D-*ribofuranosidoguanine* so obtained was identical in m. p. behaviour and optical rotation with natural guanosine, and the *X*-ray powder photographs of the natural and synthetic materials were indistinguishable. Fischer and Helferich's views on the course of the reactions involved receive confirmation from this identity.

The above synthesis gives final verification of the structures now accepted for guanosine and its deamination product, xanthosine. Furthermore, in this synthesis guanosine has been prepared from the same 2 : 8-dichloro-9-D-ribofuranosidoadenine as was employed in the synthesis of adenosine (Part XIX, *loc. cit.*). It follows, therefore, that guanosine and consequently xanthosine have the same configuration at the glycosidic centre as adenosine; we regard it as virtually certain that this is of the β-type (cf. Part XII, *J.*, 1946, 833; Part XV, *ibid.*, p. 861). This feature of the guanosine molecule, although suspected from the similarity between its optical rotation and that of adenosine, we had previously been unable to establish by direct chemical methods. It may be noted that the conclusion is now warranted that all four purine and pyrimidine nucleosides present in yeast ribonucleic acid are β-D-ribofuranosides, a fact which may be significant in determining the structural pattern of the polynucleotide.

Experimental.

Guanine Glucoside.—2 : 8-Dichloro-9-β-D-glucopyranosidoadenine (1 g., Part XII, *loc. cit.*) in dilute sodium hydroxide solution (135 c.c. of 0·037N) was shaken at room temperature with palladised barium sulphate (1·5 g. of material prepared from 22 g. of barium sulphate and 1·8 g. of palladous chloride) in an atmosphere of hydrogen. After 9 minutes the hydrogen uptake corresponded to removal of one chlorine atom, and the solution was filtered from catalyst and neutralised with dilute hydrochloric acid. Evaporation of the solution to small bulk (*ca.* 10 c.c.) gave 2-chloro-9-β-D-glucopyranosidoadenine (0·52 g.), separating from water as fine needles sintering at 190°, m. p. 223° (decomp.) (Found : Cl, 10·5. Calc. for $C_{11}H_{14}O_5N_5Cl$: Cl, 10·7%). This material was identical with that obtained by reduction with zinc dust as described by Fischer and Helferich (*loc. cit.*). Conversion into guanine glucoside was accomplished by the methods of these authors. From the above monochloroglucoside (1 g.) there was obtained a specimen of material (0·1 g.) with the following characteristics : m. p. 295—305°, $[\alpha]_D^{16°}$ − 44·6° (*c*, 0·68 in N-sodium hydroxide) (Found in material dried at 140°/0·1 mm. : C, 42·3; H, 5·3; N, 22·5. Calc. for $C_{11}H_{15}O_6N_5$: C, 42·2; H, 4·8; N, 22·4%). Fischer and Helferich record for guanine glucoside, m. p. 298°, $[\alpha]_D^{15}$ − 41·6° (*c*, 7·5 in N-sodium hydroxide). This material gave an intense colour with the phenol reagent described by Hitchings (*J. Biol. Chem.*, 1941, **139**, 845). Whilst not specific for guanine this reaction is known to fail when applied to purines containing an unsubstituted position 2 (adenine, hypoxanthine). The only possible alternative for the structure of the compound, *viz.*, 8-amino-6-hydroxypurine glucoside, would therefore be expected to give no colour when tested in this way.

Guanosine.—2 : 8-Dichloro-9-β-D-ribofuranosidoadenine (0·950 g., Part XIX, *loc. cit.*) in dilute sodium hydroxide solution (120 c.c. of 0·025N) containing palladised barium sulphate (0·7 g.) was shaken with hydrogen at room temperature until the volume corresponding to removal of one chlorine atom had been absorbed (21 minutes). After removal of catalyst and neutralisation of the solution (dilute hydrochloric acid), it was evaporated to dryness. A solution of the product in water (40 c.c.) was treated with sodium nitrite (1·37 g.) and glacial acetic acid (1·6 c.c.) and kept at 25° for 7 hours. After addition of more sodium nitrite (0·8 g.) and acetic acid (1 c.c.) the solution was allowed to stand

at 25° for a further 12 hours, and then evaporated to dryness under reduced pressure at 35°. The residue was dissolved in water and treated with lead acetate (2·57 g.) and excess of aqueous ammonia. The precipitate was collected, washed with water, dissolved in glacial acetic acid (40 c.c.), and the solution decomposed with hydrogen sulphide. After removal of lead sulphide the solution was evaporated to dryness under reduced pressure, and the residue heated at 150° for 5 hours with ethanolic ammonia (53 c.c. of ethanol containing 3·7 c.c. of water saturated with ammonia at 0°). The cooled solution was filtered, evaporated to dryness under reduced pressure, and the residue extracted with hot water (30 c.c.). The extract was treated with lead acetate (2·6 g.) and excess of aqueous ammonia, and the precipitate collected, washed, and dissolved in dilute acetic acid (40 c.c. of 4·5%). The solution was decomposed with hydrogen sulphide, the filtrate from the lead sulphide evaporated to dryness under reduced pressure, and the residue dissolved in hot water (7 c.c.). On cooling, 9-*β*-D-*ribofuranosidoguanine* separated and was purified by recrystallisation from water (5 c.c.). Yield, 72 mg.; m. p. 239° (decomp.) (rapid heating), alone or in admixture with guanosine obtained by hydrolysis of yeast ribonucleic acid. $[\alpha]_D^{18°}$ − 64° (*c*, 0·193 in 0·1N-sodium hydroxide) (Found in material dried over phosphoric oxide at room temperature/15 mm. for 48 hours: C, 37·5; H, 5·7; N, 21·8. $C_{10}H_{13}O_5N_5,2H_2O$ requires C, 37·6; H, 5·3; N, 21·9%. Found in material dried at 124°/1 mm.: N, 24·5. $C_{10}H_{13}O_5N_5$ requires N, 24·7%). A sample of natural guanosine crystallised and dried under the same conditions as above gave analytical values corresponding to the same dihydrate as recorded for the synthetic specimen (Found in material dried at room temperature/15 mm. for 48 hours over phosphoric oxide: C, 37·6; H, 5·2; N, 22·1%). Natural and synthetic samples of the dihydrate were examined by *X*-ray powder photography in the Department of Crystallography of this University and their identity confirmed.

We record our gratitude to the Department of Scientific and Industrial Research for a Maintenance Grant to J. D., to Roche Products Ltd. for gifts of material, and to Dr. J. H. Birkett Clews for the *X*-ray crystallographic examination.

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345. *Thymol and* cyclo*Hexanol as Fractionating Agents for Starch.*

By E. J. BOURNE, G. H. DONNISON, SIR NORMAN HAWORTH, and S. PEAT.

The separation of starch into its components, amylose and amylopectin, is now carried out by a standardised procedure herein described. The method involves the use of thymol as an amylose-precipitating agent, the amylopectin remaining in solution.

*cyclo*Hexanol is recommended as a fractionating agent in place of thymol when the remaining starch component, namely amylopectin, is desired in a purer condition. *cyclo*Hexanol–amylose contains a higher proportion of amylopectin than does the thymol–amylose.

It is shown that the *cyclo*hexanol–amylopectin fractions of very low blue value, reported by Haworth, Peat, and Sagrott (*Nature*, 1946, **157**, 19), are not normal constituents of starch but are hydrolytic degradation products of amylopectin. When *cyclo*hexanol–amylopectin is submitted for a short period to the action of saliva or of mineral acid, a precipitate forms in the presence of excess of *cyclo*hexanol which has the character of amylose. The amylose–*cyclo*hexanol complex is not itself susceptible to hydrolysis by either the enzyme or the acid used.

THE branched and unbranched components of starch have been separated in a number of ways which have been summarised recently by T. Schoch (" Advances in Carbohydrate Chemistry ", Vol. 1, 1945). Haworth, Peat, and Sagrott (*Nature*, 1946, **157**, 19) briefly described the effect of a number of hydroxylic conpounds which functioned as precipitants of the amylose component. Among these precipitants thymol and *cyclo*hexanol appeared to be particularly suitable as agents for the fractionation of starch. It is the purpose of this communication to give a fuller account of the experience gained during the past three years in the use of these methods by a group of workers in these laboratories who have been engaged in the study of various aspects of the chemistry and physiology of starch.

The Thymol Method.—The standard procedure now used by us is described in detail in the experimental section. It is essential that the starch be dispersed as completely as possible in boiling water to which a salt (0·1% NaCl) is usually added, and that any undispersed material be removed before the addition of thymol. The concentration of starch should not exceed 3% in the presence of ions or 1% in their absence. The precipitation of the amylose–thymol complex begins a few hours after the addition of thymol (to saturation) and is usually complete in 48 hours. It is advisable, however, to allow 60 hours to elapse before the precipitate is separated from the amylopectin-containing mother liquor. The amylose–thymol complex is obtained in a more readily separable form if it is precipitated at a slightly elevated temperature (30°). The amylose precipitate is best freed from residual amylopectin solution by being repeatedly washed with water saturated with thymol.

The optimum conditions prescribed above were determined by a systematic variation of the following factors: the initial concentration of the starch; the viscosity and the pH of the starch

dispersion; the temperature at which the precipitation of the amylose–thymol complex is allowed to proceed; and the time elapsing between the addition of the thymol and the removal of the precipitate. The most important factor appears to be the physical state of the initial dispersion of the starch in water, and this cannot be always rigidly controlled. A further complication is due to the colloidal instability of the unbranched amylose component which is often precipitated spontaneously as an unmanageable gel, enclosing within its substance a relatively large volume of an aqueous solution of the amylopectin component. The larger the scale on which the separation is carried out the greater is the tendency of the partly purified amylose to form such a gel. This tendency appears to be largely conditioned by the degree of dispersion of the original starch paste which in turn depends upon the concentration of the starch and the duration and efficiency of the stirring of the boiling paste.

Some representative results of the separation of the components of potato starch by the use of thymol are shown in Table I. The blue value (B.V.) of the iodine complex and the extent of

TABLE I.

Specimen results by the standard thymol method.

Separation No.	Variations from standard method.	Amylose fraction: yield (%).	B.V.	Amylopectin fraction, B.V.
I	None	23·0	1·15	0·220
II	,,	18·4	1·17	—
III	,,	18·9	1·21	0·230
IV	,,	20·5	1·16	0·211
V	Centrifuging (25,000 r.p.m.) omitted	—	1·12	—
VI	,, ,, ,,	20·0	1·11	—
VII	Precipitation at room temperature (5—20°)	21·0	1·05	0·250
VIII	,, ,, ,, ,,	15·3	1·06	0·168
IX	,, ,, ,, ,,	25·2	1·01	—
X	Washing with thymol-water omitted	18·6	1·03	—
XI	,, ,, ,,	27·7	1·01	—
XII	,, ,, ,,	20·1	0·95	—
XIII	,, ,, ,,	14·2	0·83	—
XIV	Sodium chloride omitted	No separation		

hydrolysis effected by β-amylase are taken as criteria of the degree of separation of the amylose from the amylopectin component. Average specimens of " thymol "–amylose and –amylopectin from potato starch show blue values of 1·0—1·2 and 0·21—0·23 and are hydrolysed by β-amylase to the extent of 96—99% and 47—62% respectively.

cyclo*Hexanol as an Amylose Precipitant.*—The fractionation of potato starch by precipitation of the amylose–*cyclo*hexanol complex yields fractions which are slightly different in composition from those usually obtained by the thymol method. Without at this stage discussing the question of the chemical homogeneity of the components of starch, it is obvious that none of the methods employed yields a " pure " amylose or a " pure " amylopectin, but rather that a given reagent (*e.g.*, thymol) separates starch into two fractions each of which may be regarded provisionally as a mixture of an amylose which, by definition, consists entirely of long glucose chains without cross linkages, and an amylopectin in which no long unbranched chains are present. To distinguish the fractions actually obtained in a given separation from these hypothetical entities, we have adopted the system of incorporating the name of the fractionating agent in the description of the fractions (*e.g.*, thymol–amylose, *cyclo*hexanol–amylopectin, etc.) whenever clarity demands such a distinction.

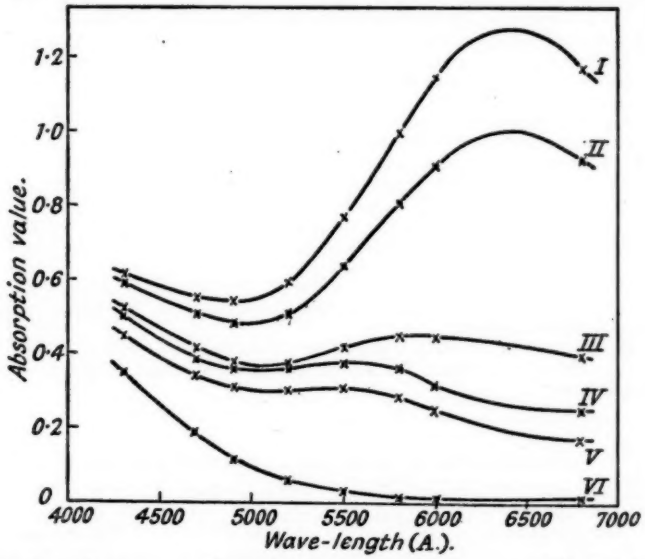
The blue values of average specimens of thymol–amylose and *cyclo*hexanol–amylose (each from potato starch) are 1·1 and 0·9 respectively, the corresponding amylopectins showing B.V., 0·22 and 0·18. For a compariosn of two methods of fractionation it is legitimate to assume that the higher the B.V. of a starch-component the higher the amylose- and the lower the amylopectin-content. On this basis, it is evident that *cyclo*hexanol yields a purer amylopectin than does thymol, whereas thymol gives an amylose containing less of the branched-chain constituent than occurs in *cyclo*hexanol–amylose.

If the absorption values (A.V.) of a standard solution of polysaccharide and iodine are measured using a range of Ilford filters it is possible to plot A.V. against wave-lengths of peak transmission of the filters. It will be convenient to describe such a graph as an absorption curve. The components of starch can be distinguished by the forms of their absorption curves. This is illustrated in the figure, which also shows that the absorption by thymol–amylose is greater at all wave-lengths than that by *cyclo*hexanol–amylose.

Haworth, Peat, and Sagrott (*loc. cit.*) occasionally obtained specimens of *cyclo*hexanol–amylopectin with very low blue values (*ca.* 0·02). Such specimens could never be produced at will, and subsequent work demonstrated that they were not normal constituents of starch but were formed, possibly because of contamination by traces of saliva, by hydrolysis of the true *cyclo*hexanol–amylopectin (B.V., 0·18).

In the course of experiments designed to test the action of salivary amylase in high dilution on amylopectin in the presence of *cyclo*hexanol there was invariably observed the separation of a small precipitate which was stained blue by iodine and showed other properties which classified it as of the amylose and not of the amylopectin type. Control experiments established that this was a true enzyme action and that partial hydrolysis of the amylopectin was a prerequisite for the precipitation of the intermediate amylose fraction. It is interesting to note that the insoluble *cyclo*hexanol–" amylose " complex appears not to be itself susceptible to hydrolysis by the amylase. It was later shown that any starch-hydrolysing agent could effect the separation of the intermediate amylose fraction from amylopectin in the presence of *cyclo*hexanol. Thus, when an amylopectin dispersion was heated for 3 minutes in a boiling water-bath with N-sulphuric

Specimen light-absorption curves of polysaccharide iodine complexes.



I, *Thymol–amylose.* II, cyclo*Hexanol–amylose.* III, *Potato starch.*
IV, *Thymol–amylopectin.* V, cyclo*Hexanol–amylopectin.* VI, I_2–KI *alone.*

acid in the presence of *cyclo*hexanol, a flocculent precipitate was thrown down. This product, which was obtained in 1·5% yield, showed a higher B.V. (0·355) than that of the original amylopectin (B.V., 0·210).

Pacsu and Hiller (*Text. Research J.*, 1946, **16**, 243) have also observed the liberation of an " amylose " component when an amylopectin (*e.g.*, waxy maize) is treated either with 0·1N-sulphuric acid or with α-amylase. It is difficult, however, to reconcile the evidence available with the novel view of these authors that the observation just recorded represents the conversion of a branched amylopectin structure into an amylose with fewer branches.

Experimental.

Absorption Value (A.V.) and Blue Value (B.V.).—The expression " absorption value " is used to describe the reading on the logarithmic scale of a Spekker Photoelectric Absorptiometer when the absorption of light by a solution of a polysaccharide–iodine complex, contained in 4 cm. cells, is measured. The light filters employed were of the Ilford gelatin type (Nos. 601—608, transmitting light of 4300—6800 A.).

The term " blue value " has a special significance; it is the A.V. observed when the polysaccharide–iodine solution is prepared under standard conditions and when Ilford gelatin light filters transmitting light of 6800 A. are employed. Details for the preparation of a standard solution of this type were given by Bourne, Haworth, Macey, and Peat (this vol., p. 924).

Hydrolysis of Starch and its Components.—(a) *By aqueous acid.* The polysaccharide, after being dried

in a vacuum at 60° for about 6 hours, was weighed into a boiling tube and heated under reflux in a boiling water-bath with 7% sulphuric acid for 12 hours. The hydrolysate was then cooled, neutralised with sodium hydroxide, and diluted to 100 c.c. The amount of glucose present in the neutral solution was determined by means of the Shaffer–Hartmann copper reagent (see Bourne, Haworth, Macey, and Peat, *loc. cit.*).

As amylose is only sparingly soluble in water it is advisable to dissolve it initially in 2% sodium hydroxide (10 c.c.) and then add sulphuric acid to give a final volume of 25 c.c. with an acid concentration of 7%

(b) *By β-amylase.* A general method was adopted for the measurement of the extent of hydrolysis of a 1:4-α-glucosidic polysaccharide effected by the β-amylase of soya bean (Bourne, Macey, and Peat, *J.*, 1945, 882). A digest consisting of the dry polysaccharide (about 30 mg.) in water (34 c.c.), acetate buffer (pH 4·8; 6 c.c.), and β-amylase solution (10 c.c.; 0·1%) was covered with a layer of toluene and incubated at 35·5°. At frequent intervals the amount of maltose formed was determined by removing aliquot portions for analysis by the Shaffer–Hartmann method.

When the polysaccharide was not readily soluble in warm water, it was dissolved by warming with 2% sodium hydroxide solution (10 c.c.), the solution being accurately neutralised by the addition of 5N-sulphuric acid and the volume being adjusted to 34 c.c. with water.

A control digest, containing all the ingredients except the polysaccharide, was used in each determination. Only a small reducing power, if any, was developed in these controls. Where necessary a small correction was applied on this account.

Standard Procedure for the Fractionation of Potato Starch by the Thymol Method.—Potato starch (120 g., dry weight) suspended in cold water (500 c.c.) was slowly added, with continuous stirring, to boiling water (3500 c.c.). The viscosity of the paste was lowered by adding sodium chloride (4 g.) and continuing to stir the boiling solution for 20 minutes. During this process the maximum efficiency of stirring should be achieved. The paste was cooled to 70° and then passed through a Sharples supercentrifuge (25,000 r.p.m.) at a rate of 4 l. per hour. Any small undissolved residue which was thereby removed was discarded.

The temperature of the paste was adjusted to 30°, and powdered thymol (8 g.) was added, with stirring. The mixture was kept at 30° for 60 hours, during which time the insoluble amylose–thymol complex settled out. This was removed in a centrifuge (2000 r.p.m.), washed by being stirred with water saturated with thymol (1 l.), and again separated. The washing process was repeated with 2 fresh portions of water saturated with thymol. The precipitate was dehydrated and freed from thymol by being triturated repeatedly with alcohol and finally with ether. It was dried in a vacuum over phosphoric oxide. The treatment with alcohol and then with ether removed all but a trace of thymol from the complex.

The amylopectin fraction was precipitated by the addition of alcohol (4500 c.c.) to the concentrated mother liquor (1500 c.c.) after the removal of the amylose complex. It was washed with alcohol and ether and dried over phosphoric oxide.

The method as described is suitable for quantities of starch not greater than 150 g. On a bigger scale, *e.g.*, on 1 kg., complete dispersion of the starch in water requires a longer period (up to 4 hours) of stirring at the boiling point.

TABLE II.

β-Amylolysis of potato starch and its fractions.

Thymol separation No. (See Table I).	B.V. of polysaccharide.	Conversion into maltose (%).	Thymol separation No. (See Table I).	B.V. of polysaccharide.	Conversion into maltose (%).
Amylopectin :			Amylose :		
VIII	0·168	49·2	X	1·03	81·4
I	0·220	62·0	VIII	1·06	92·2
VII	0·250	47·0	V	1·12	99·0
Starch :	0·400	60·2	I	1·15	98·2
			III	1·21	96·8

Effect of Various Factors on the Degree of Separation of Amylose and Amylopectin Achieved by the Thymol Method.—(a) *Hydrogen-ion concentration.* An approximately 2% dispersion of potato starch was prepared and divided into portions (100 c.c.). To each portion were added, with stirring, a buffer solution (100 c.c.), thus reducing the starch concentration to 1%, and powdered thymol (2 g.). A range of buffer solutions was prepared from the Universal Buffer containing phosphoric, acetic, and boric acids (see Britton's " Hydrogen Ions "). After each mixture had been kept at 30° for 48 hours the amylose and amylopectin fractions were isolated as in the standard method.

TABLE III.

Effect of pH *on the fractionation of starch.*

pH of buffer.	Amylose fraction. Yield (%).	Amylose fraction. B.V.	Amylopectin fraction, B.V.	pH of buffer.	Amylose fraction. Yield (%).	Amylose fraction. B.V.	Amylopectin fraction, B.V.
5·0	27	0·96	0·21	8·0	24	1·03	0·19
6·0	24	0·93	0·21	9·0	27	0·95	0·19
7·0	26	0·96	0·19	10·0	27	0·89	0·19
				11·0	27	0·90	0·20

Since no attempt was made to remove inorganic matter from the products the above figures are only approximate. They are, however, sufficiently accurate to indicate that any variations attributable to pH changes must be very slight.

(b) *Concentration of the starch paste.* Aqueous pastes (100 c.c.) were prepared containing 0·25, 0·5, 1·0, 2·0, and 5% of potato starch. Into each was stirred powdered thymol (2 g.), and the mixtures were kept at 30° for 48 hours before being centrifuged (2000 r.p.m.). The amylose–thymol complex was readily separated in the first three cases, but sedimentation was slow and incomplete from the 2% and 5% pastes. Thus it appeared that starch pastes of 1% or less would be the most suitable for quantitative separation. The difficulties encountered with the more concentrated pastes were attributed to their higher viscosities (no salt was added in these experiments), which prevented the sedimentation of the fine particles of the thymol–amylose complex.

(c) *Initial viscosity of the starch paste.* Four pastes (200 c.c.) (3%) of potato starch were prepared in the usual manner and, after being cooled, were " thinned " by the addition of sodium chloride (0·1%). Traces of insoluble matter were removed by centrifuging. Although these pastes had been prepared under similar conditions they differed appreciably in their relative viscosities as measured by an Ostwald viscometer. Into each paste was stirred powdered thymol (2 g.), and the mixture was kept for 48 hours at 30°. The precipitated amylose–thymol complex was collected in a centrifuge (2000 r.p.m.), and washed 4 times with saturated thymol-water (150 c.c. each), and then with alcohol (twice) and ether (twice).

The amylopectin was recovered from the supernatant liquid by precipitation with alcohol, and washed with alcohol (twice) and ether (twice). Both fractions were dried in a vacuum over phosphoric oxide. The degree of separation achieved with pastes of different viscosity is shown in Table IV.

TABLE IV.

Initial η_r of paste (water = 1).	Blue values.	
	Amylose fraction.	Amylopectin fraction.
83·5	No separation	
25·9	0·84	0·24
15·3	1·03	0·22
12·4	1·10	0·21

In another experiment, a 3% paste of potato starch was prepared and, when cold, sodium chloride (0·1%) was added. The resultant paste, which had $\eta_r = 50·2$, was divided into 4 parts, which were respectively (i) vigorously stirred at room-temperature for 3 hours, (ii) boiled for 20 minutes, (iii) diluted with an equal volume of water to give a 1·5% paste, and (iv) diluted with water to give a 1% paste. The 4 solutions were then subjected to fractionation with thymol by the method already outlined, with the results shown in Table V.

TABLE V.

No.	Initial η_r of paste (water = 1).	Blue values.	
		Amylose fraction.	Amylopectin fraction.
(i)	45·8	No separation	
(ii)	13·3	0·89	0·24
(iii)	5·7	0·94	0·17
(iv)	2·7	1·02	0·16

In a third experiment, a potato starch paste (6%) was divided into 3 portions which were treated with alkali, respectively, as follows :

(i) diluted with sodium hydroxide solution to give a paste which contained 3% of starch and 2½% of sodium hydroxide ;

(ii) made 2½% with respect to sodium hydroxide, stirred at room temperature for 1 hour, neutralised with hydrochloric acid, and diluted with water to give a 3% starch paste ;

(iii) as under (ii), except that the temperature was 50° during the stirring process.

The 3 solutions were then subjected to fractionation with thymol with the results shown in Table VI.

TABLE VI.

No.	Initial η_r of paste (water = 1).	Blue values.	
		Amylose fraction.	Amylopectin fraction.
(i)	19·9	1·00	0·21
(ii)	11·2	1·04	0·24
(iii)	8·5	1·14	0·27

The more drastic the treatment with alkali the more difficult it became to remove the finely-divided amylose complex from the amylopectin solution. Hence the B.V. of the latter increased. However, when the complex was removed in the Sharples supercentrifuge in case (iii) the B.V. of the amylopectin was as low as 0·16.

(d) *Temperature and duration of complex formation.* These factors were not specifically controlled, but the results of several workers indicated that the optimum conditions were those reported in the standard method.

(e) *Treatment of the components with thymol-water.* (i) *Amylose.* A sample of a moist amylose–thymol complex isolated from potato starch was divided into 3 parts. The first part was washed with saturated

thymol-water (3 volumes), the second was washed twice in this way, while the third was not treated. All were washed with alcohol and then ether before being dried in the usual manner. The B.V. of the unwashed material was 1·04, which was increased by the first washing to 1·11 and by the second washing to 1·14.

(ii) *Amylopectin.* A sample of potato amylopectin (3 g.; B.V., 0·240) was shaken at room temperature for 18 hours with saturated thymol-water (100 c.c.). The insoluble residue was removed by centrifuging and discarded. The soluble polysaccharide, which was precipitated by the addition of alcohol, washed with alcohol and then ether, and dried, had B.V., 0·235. A second sample (3 g.) of the same amylopectin which was shaken with saturated thymol-water for 42 hours gave a product having B.V., 0·222. A third sample (3 g.) of the amylopectin was heated at 100° for 2 hours with saturated thymol-water; the product had B.V., 0·221. When the heating at 100° was continued for 12 hours the product had B.V., 0·219.

The Fractionation of Potato Starch by the cyclo*Hexanol Method.*—The general procedure used in these experiments was similar to that recorded for the standard thymol method. The amounts of *cyclo*hexanol used instead of thymol are recorded in Table VII, where a summary of a number of fractionations is given. The amylose fractions were, of course, washed with saturated *cyclo*hexanol–water instead of thymol-water.

TABLE VII.

Specimen results by the cyclo*hexanol method.*

Separation No.	Starch paste (%).	*cyclo*Hexanol c.c./100 c.c. of paste.	Amylose fraction. Yield (%).	Amylose fraction. B.V.	Amylopectin fraction, B.V.
I	3	0·2	37·6	0·72	0·225
II	3	5·0	43·1	0·85	0·220
III	3	5·0	—	—	0·156
IV	1	0·5	30·8	0·93	0·096
V	1	0·5	24·0	0·93	—
VI	1	1·0	23·9	0·93	—
VII	1	2·0	27·2	0·91	—
VIII	1	3·0	27·9	0·94	—
IX	1	4·0	27·0	0·89	0·218
X	1	5·0	23·0	0·94	—
XI	1	6·0	23·8	0·95	—
XII	1	6·0	25·1	0·91	0·094
XIII	1	6·0	—	—	0·193
XIV	1	6·0	—	—	0·182
XV	1	6·0	—	—	0·152

Action of Saliva on Potato Amylopectin in Presence of cyclo*Hexanol.*—A 1% paste of potato starch, to which a trace of sodium chloride had been added, was allowed to stand for 3 days at 30° in the presence of sufficient *cyclo*hexanol to maintain a saturated solution throughout this period. The insoluble *cyclo*hexanol–amylose complex was removed by passing the suspension thrice through a Sharples supercentrifuge (25,000 r.p.m.). Portions (10 c.c.) of the amylopectin solution, still saturated with *cyclo*hexanol, were incubated at 25° with mucin-free saliva (1 c.c.) under different conditions. The precipitated fraction, if any, was separated (2000 r.p.m.), washed twice with alcohol, then with ether, and dried. Its staining power with iodine was tested qualitatively.

A blue-staining precipitate was isolated in several experiments in which untreated mucin-free saliva was used, but there was no precipitate when the saliva had been pre-heated for 10 minutes at 100° or when mercuric chloride (1 part in 1000), which inhibits α-amylase action, was incorporated in the digest.

Effect of Salts on the α-Amylolysis of Potato Amylopectin in Presence of cyclo*Hexanol.*—A paste (0·75%) of potato amylopectin (B.V., 0·210) was covered with a layer of *cyclo*hexanol and kept for 3 days at 30°. All ions were removed from this solution and from mucin-free saliva by passage through ion-exchange resins. Two digests were prepared containing the following constituents:

Digest (A): ion-free amylopectin paste (30 c.c.), ion-free saliva (1 c.c.), and distilled water (8 c.c.).

Digest (B): ion-free amylopectin paste (30 c.c.), ion-free saliva (1 c.c.), distilled water (2 c.c.), and acetate buffer (pH 4·8) (6 c.c.).

The buffer (pH 4·8) was chosen because the natural pH of digest (A) was 4·8. Each digest was incubated at 25°, and at intervals portions (3 c.c.) were withdrawn, immersed in a boiling water-bath for 5 minutes to destroy α-amylolytic activity, and centrifuged. A portion (1 c.c.) of the supernatant layer was introduced into a 500 c.c. flask containing water (200 c.c.), 5N-hydrochloric acid (3 drops), and iodine solution (5 c.c.; 0·2% iodine in 2% potassium iodide solution). After dilution to 500 c.c. the A.V. (6800 A.) was measured. The results are recorded in Table VIII.

TABLE VIII.

Time of incubation (mins.).	Absorption values (6800 A.). (A).	(B).
0	0·31	0·31
60	0·31	0·05
120	0·31	0·03

It was observed that a blue-staining precipitate separated from digest (B), but not from digest (A), in which no enzyme action occurred.

Fractionation of Potato Amylopectin with cyclo*Hexanol and Saliva.*—Potato amylopectin (2·8 g.), dispersed in water (400 c.c.), was covered with a layer of *cyclo*hexanol and kept at 30° for 3 days. It was heated to 40° and mucin-free saliva (5 c.c.) was added. The solution was immediately heated, and in 35 seconds reached 100°, and was kept there for 10 minutes. The precipitated polysaccharide was separated (2000 r.p.m.), washed, twice with alcohol and twice with ether, and dried in a vacuum over phosphoric oxide. Yield, 56 mg.; B.V., 0·66; 94·5% hydrolysed by β-amylase (mean).

It thus appeared to be a degraded amylose type.

Fractionation of Potato Amylopectin with cyclo*Hexanol and Acid.*—Potato amylopectin (1·50 g., B.V., 0·210) was dispersed in water (200 c.c.), covered with a layer of *cyclo*hexanol, and kept at 30° for 3 days. Sulphuric acid (5N; 50 c.c.) was added, making a N-acid solution, and, still covered with *cyclo*hexanol, it was immersed in a bath of vigorously boiling water. After the solution had been above 95° for 3 minutes, it was cooled and centrifuged (2000 r.p.m.). The precipitate was washed thrice with alcohol and then with ether before being dried in a vacuum over phosphoric oxide. Yield, 23 mg.; B.V., 0·355.

The Light-absorption Curve of a Polysaccharide–Iodine Complex.—The dry polysaccharide was dissolved and stained with iodine under the standard conditions prescribed by Hassid and McCready (*J. Amer. Chem. Soc.*, 1943, **65**, 1154), giving a coloured solution which contained the polysaccharide (1 mg./100 c.c.), iodine (2 mg./100 c.c.) and potassium iodide (20 mg./100 c.c.). The absorption values of this solution were determined over the whole range of Ilford gelatin filters (Nos. 601—608). By plotting A. V. against the wave-length of peak filter transmission (4300—6800 A.), a characterstic "absorption curve" for the polysaccharide was obtained. This curve of course incorporated the B.V. at 6800 A.

We wish to acknowledge the contributions made to the results quoted here by other members of this research group, namely, by P. E. Sagrott, R. D. Thrower, and W. J. Whelan.

We are grateful also to the Colonial Products Research Council for financial assistance.

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346. *A Study of Bituminous Coal by* (a) *Exhaustive Solvent Extraction and* (b) *X-Ray Diffraction.*

By H. E. Blayden, J. Gibson, and H. L. Riley.

A semimicro-method for the exhaustive solvent extraction of coal with pyridine and chloroform is described. This method has been used in a detailed study of the effect of carbonisation and oxidation on the yield of α (pyridine-insoluble), β (pyridine-soluble, chloroform-insoluble), and γ (pyridine- and chloroform-soluble) fractions. The three fractions from the unoxidised and oxidised coal and the chars and cokes obtained from them at various temperatures have been examined by *X*-ray powder photography, and the results obtained provide further evidence in support of the views already advanced concerning the importance of the proportion of oxygen in determining the crystallographic behaviour of a carbonaceous substance on carbonisation. Although the caking properties of the original coal were completely destroyed by the oxidation, the γ extract from the oxidised coal still caked strongly on carbonisation.

Previous investigations ("The Ultra-Fine Structure of Coals and Cokes", B.C.U.R.A., London, 1944, 176—231; *J. Inst. Fuel*, 1945, War Time Bulletin, 117) have led us to the view that bituminous coal consists essentially of two related, but crystallographically distinct substances, *viz.*, (*a*) bitumen and (*b*) residual coal. The bitumen is that part of the coal which can be extracted by organic solvents: a greater or a lesser part of the bitumen, according to the rank of coal, consists of thermally stable, polynuclear, aromatic molecules which only lose their individuality and become converted into "carbon" at temperatures in the 400—550° range. It is this part of the bitumen which confers on a coal its coking properties. The remainder of the bitumen consists of waxy, resinic, and naphthenic substances, some of which may be converted into coking bitumens during carbonisation (*Nature*, 1947, **159**, 536). Much less is known of the molecular structure of the residual coal: it contains a higher proportion of oxygen and a lower proportion of hydrogen and is thermally less stable than the coking bitumens, and its crystallographic behaviour on carbonisation indicates that it has a disordered crystal structure which is either actually or potentially cross-linked. It is probably related structurally to the humic acids and the humins.

Pyridine is known to have a high solvent and peptising action on bituminous coal. According to Wheeler and his co-workers (see Bakes, D.S.I.R. Fuel Research Tech. Paper No. 37, 1933, for references and review of work on solvent extraction of bituminous coal), the insoluble residue (α-fraction) of the pyridine extraction of a bituminous coal consists of undispersed ulmins together with resistant plant remains: the pyridine-soluble, chloroform-insoluble, β-fraction consists of dispersed ulmins, and the pyridine-soluble, chloroform-soluble γ-fraction consists of saturated and unsaturated hydrocarbons together with resinous materials.

The objectives of the present investigations were (*a*) to determine how far it is possible to correlate the results of the exhaustive pyridine extraction of a coking coal and of cokes prepared from it, with the results of *X*-ray investigations already reported, and (*b*) to investigate the change in the *X*-ray crystallographic properties which the α-, β-, and γ-fractions undergo when the caking properties of the original coal are destroyed by oxidation. In spite of the high solvent and peptising action of dry pyridine on bituminous coal, exhaustive extraction on the macro-scale is a lengthy and tedious operation: the following semimicro-procedure was therefore adopted.

A sample of the coal (about 0·02 g.), freshly crushed to pass a 240 B.S. sieve, was placed in a centrifuge tube (30 ml.) of known weight, and dried in a stream of dry, oxygen-free nitrogen at 120°. The centrifuge tube and contents, contained in a stoppered weighing tube, were weighed. Technical pyridine was dried over potassium hydroxide and redistilled, the fraction of b. p. 120—130° being collected and redistilled as required, immediately before use. The dry pyridine (3 ml.) was added to the coal in the centrifuge tube and then heated for 10 mins. under

FIG. 1.

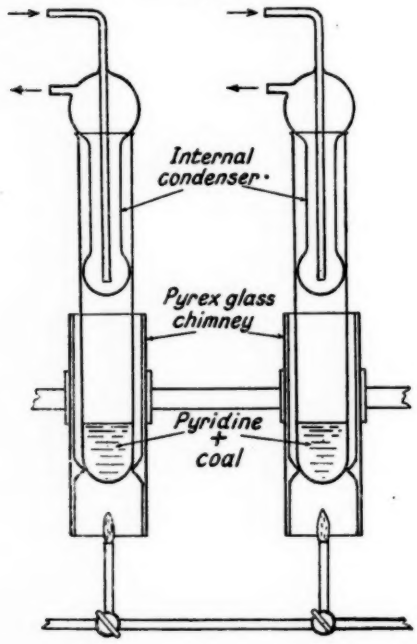
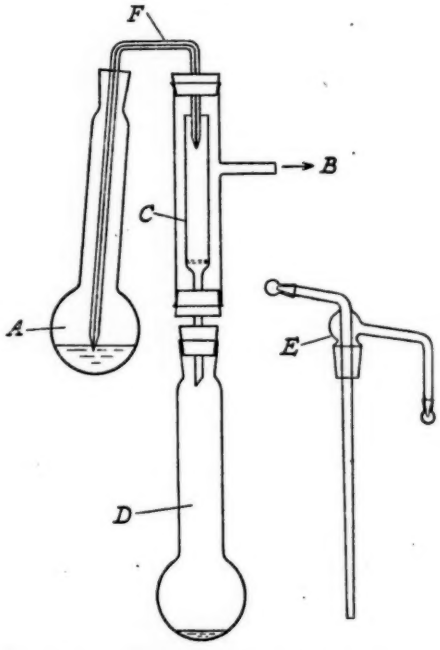


FIG. 2.

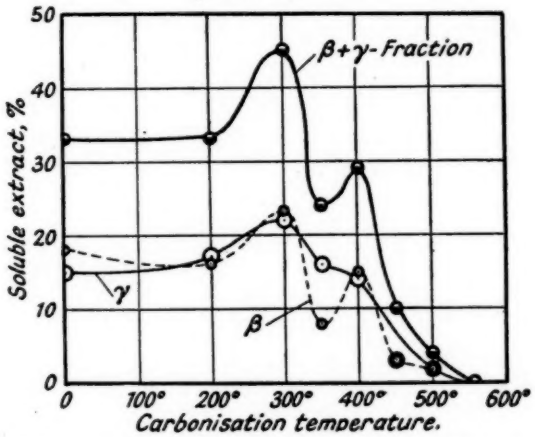


a reflux condenser which was formed by inserting a water-cooled tube in the top part of the centrifuge tube (see Fig. 1): ingress of air was minimised by the formation of a liquid pyridine seal between the condenser wall and the centrifuge tube. A battery of such tubes was erected in order to permit several semimicro-extractions to be carried out simultaneously. After about 10 mins.' extraction the tube and contents were centrifuged, the supernatant liquid was removed by means of a suction tube, a second 3 ml. of pyridine added, and the refluxing and separation repeated. These operations were continued until the supernatant pyridine, after centrifuging, was colourless: some 3—20 extractions were necessary according to the character of the sample under investigation. The extracts were combined and the pyridine removed by distillation in a stream of carbon dioxide at low pressure (5—10 cm. Hg), followed by steam-distillation for 20 mins.: residual water was then removed in a current of dry carbon dioxide at low pressure, and the product finally dried in a current of dry nitrogen at atmospheric pressure. The distillation was carried out without transference of the solution or residue from the tared distillation flask, and the final weighing gave the percentage of pyridine-soluble matter (β + γ-fraction) in the coal.

Redistilled chloroform was purified further by passage through a column of activated alumina (14—25 B.S. sieve size, freshly heated to 150°, and cooled in a stream of dry air) 2 cm. in diameter and 25 cm. long; 10 ml. of this chloroform left no perceptible residue on evaporation.

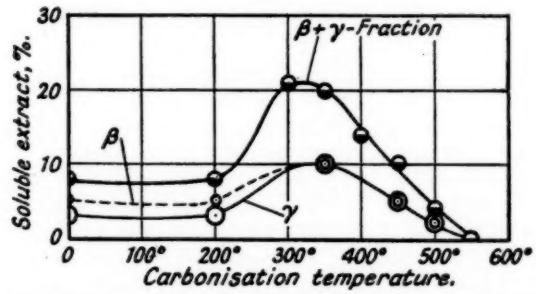
The $\beta + \gamma$-fraction in flask *A* (Fig. 2) was extracted by warming it with 2 ml. of pure chloroform which refluxed from the cold neck of the flask. The resulting liquid was drawn by suction (at *B*) into a sintered-glass micro-filter *C*. The residue in *A* was extracted with successive amounts of chloroform until a colourless extract was obtained: usually three extractions sufficed. The apparatus was then evacuated through *B*, with *F* closed; air was then allowed to enter and thus forced the chloroform solution from *C* into the second small distilling flask *D*, which was provided with a detachable distillation head *E*. This procedure was repeated if necessary until filtration was complete. The insoluble residue on the filter was well washed with pure chloroform which was introduced as described above. Flask *B* was attached to the distillation head, *E*, and the chloroform removed from the combined filtrate and washings by distillation in a stream of carbon dioxide at reduced pressure and a temperature not exceeding

FIG. 3.



Solvent extraction of Durham coal C.383 before and after preheating.

FIG. 4.



Solvent extraction of S. Wales coal C.906 before and after preheating.

100°. The last traces of chloroform were removed from the residue by heating it in a stream of carbon dioxide at low pressure (2—5 cm. Hg) for a further 15 mins. after the chloroform had ceased to distil. When the flask was cool, pure dry nitrogen was passed through it for 20 mins. and it was then stoppered and weighed. The method is probably capable of improvement: the results obtained for the proportion of γ-fraction were probably reproducible to $\pm$ 1%.

Solvent Analysis of a Durham and a South Wales Coking Coal.—It has already been shown (Blayden, Gibson, and Riley, *loc. cit.*) that if a bituminous coal is subjected to a step-wise carbonisation, the half-peak widths of the inner intense halo in the *X*-ray powder photographs of the products show systematic variations with carbonisation temperature. The *c* dimension (*i.e.*, the height) of the average, hypothetical, cylindrical, graphite-like crystallite, calculated from the half-peak width, and plotted against carbonisation temperature, passes through a more or less pronounced maximum in the neighbourhood of 550° (see Fig. 6) (experimentally, a progressive sharpening of the inner halo, up to 550° followed by a broadening). Evidence has

been put forward which suggests that this phenomenon is due to some type of unidimensional crystallisation of the mobile thermally stable bitumen molecules. It is important to determine whether or not this unidimensional crystallisation involves merely the ordering of bitumen molecules (cf. experiments on the " annealing " and carbonisation of disordered dibenzanthrone crystals already reported, *loc. cit.*), or whether it involves the progressive conversion of bitumen molecules into rigid " carbon " as the temperature is increased up to 550°.

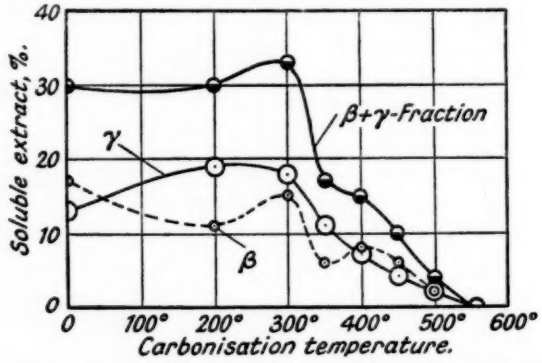
TABLE I.

Analyses of Durham and South Wales coals.

	Ash, %.	Moisture, %.	Volatile matter less moisture, %.	Volatile matter, % (a.f.d.).	B.S. swelling no.	C, % (a.f.d.).	H, % (a.f.d.).
Durham coal C.383	2·9	0·9	26·6	27·6	0	88·5	4·8
,, ,, ,, (oxidised)	—	—	—	—	9	84·4	4·29
S. Wales coal C.906	2·6	1·0	18·5	19·2	82	90·4	4·6

Samples of the two coals and their semi-cokes (prepared from C.383 by carbonising in nitrogen at 2° per min. and soaking at the final temperature for 10 mins., and from C.906 by carbonising at 5° per min. and soaking for 10 mins.) were subjected to the semimicro-solvent extraction described above. Perhaps the most significant feature of the results obtained (see Fig. 3 and 4)

FIG. 5.



Solvent extraction of the oxidised Durham coal C.383 before and after preheating.

is that the soluble bitumens have completely disappeared at a temperature near 550°—either through volatilisation or by decomposition into insoluble " carbon ". The decrease in the proportion of $\beta + \gamma$-fraction as the temperature increases from 300° to 550° suggests that the change is progressive, the less stable bitumens being converted into " carbon " at the lower temperatures. The temperature of complete disappearance of the $\beta + \gamma$-fraction is coincident with the temperature of the maximum on the *c* dimension carbonisation temperature curve (see Fig. 6): this result provides further evidence in support of the interpretation of the *X*-ray diffraction phenomenon already put forward. The pronounced increase in the proportion of $\beta + \gamma$-fraction brought about by preheating in the temperature range 200—300° is interesting. Berry (results quoted by Cockram and Wheeler, *J*, 1927, 713) has also found that preheating various coals to 200°, 250°, and 350° causes a progressive increase in the proportion extracted by either chloroform or benzene. This phenomenon, which apparently affects the β- and the γ-fraction to a similar extent, may be due to the cracking of complex aggregates or possibly to the desorption of the bitumen molecules from the α-carbon. The large difference in the proportion of soluble matter in the two coals, which have similar swelling properties, is interesting and is probably wholly or partially due to the higher rank of the S. Wales coal.

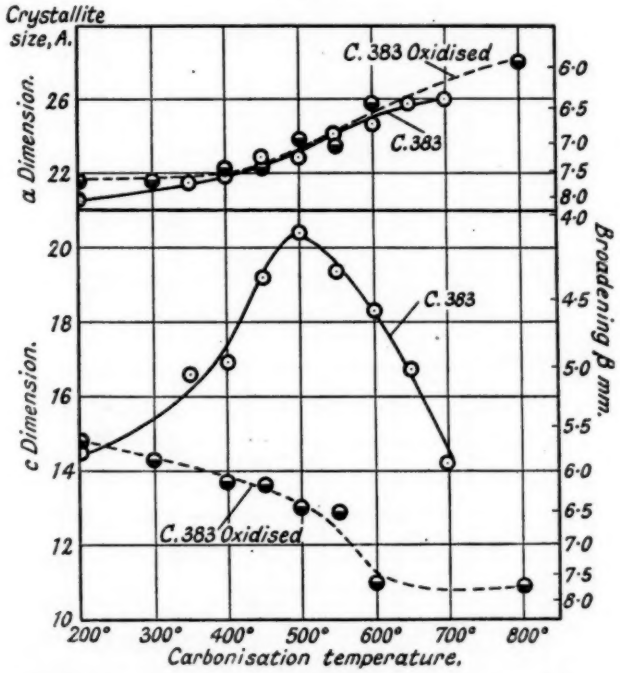
Fig. 5 shows the results of solvent extraction experiments on the Durham Coal C.383 after it had been heated in air at 120° for 60 hours, its caking and swelling power thereby being completely destroyed. This pretreatment, which brings about a fundamental change in the character of the coal, causes surprisingly little change in the general behaviour of the coal to pyridine and chloroform. The original coal, C.383, contained about 15% of γ-fraction, whereas the oxidised coal, which on carbonisation gave a non-coherent powder, still contained about 13% of

γ-fraction. Fig. 6 shows the profound effect of oxidation on the *X*-ray crystallographic behaviour: whereas the original coal gave a pronounced peak at 500° in its *c* dimension curve, the oxidised coal gave a *c* dimension which decreased with increase of the carbonisation temperature up to 600°. The *a* dimensions (diameter of the hypothetical cylindrical crystallite) of the oxidised coal and of the products obtained by heating it in nitrogen at 5° per min. up to various temperatures, showed little difference from the results obtained with the unoxidised coal.

The above observations suggested that interesting results would be obtained by separating both the fresh and the oxidised coal into α-, β-, and γ-fractions on a macro-scale, and comparing the crystallographic behaviour of the corresponding fractions on carbonisation.

The coal was ground in a ball-mill for 24 hrs. and the air-dry product (20 g., 1·5% moisture) was heated with redistilled pyridine (200 ml.) in a 500-ml. round-bottom flask provided with an internal reflux condenser. A non-oxidising atmosphere was maintained by passing a current of

FIG. 6.



town gas through the flask by way of a tube sealed in the neck of the flask. After being heated for 12—24 hrs. the contents of the flask were allowed to cool and to stand until the finely divided coal had settled: the supernatant liquid was syphoned off and filtered through a No. 4 sintered-glass filter. The residue on the filter was returned to the flask. Fresh pyridine (200 ml.) was added and the extraction repeated: this treatment was continued until the final extract was nearly colourless. Six extractions were carried out simultaneouly and occupied some 8—10 weeks. The combined extract was concentrated by distilling off the pyridine at 100° in a current of dry carbon dioxide at reduced pressure: the pyridine was finally eliminated as completely as possible by further low-pressure evaporation in carbon dioxide followed by steam-distillation for about 2 hrs. The product ($\beta + \gamma$-fraction) was dried at 100° in dry carbon dioxide and then weighed.

Pure chloroform (25 ml.) was added to the $\beta + \gamma$-fraction and the mixture refluxed on the water-bath. The resulting solution was removed by suction through a sintered-glass filter and collected in a second distilling flask, the solid remaining on the filter being returned to the original flask. The treatment was repeated until the final extract was colourless. The chloroform-soluble γ-fraction was isolated by evaporating the chloroform from the combined extracts, in a stream of dry carbon dioxide at 100°. Typical yields from 120 g. of air-dry coal were: ($\beta + \gamma$)-fraction, 16·9 g. (14%); γ-fraction 5·2 g. (4·3%). These are much less than

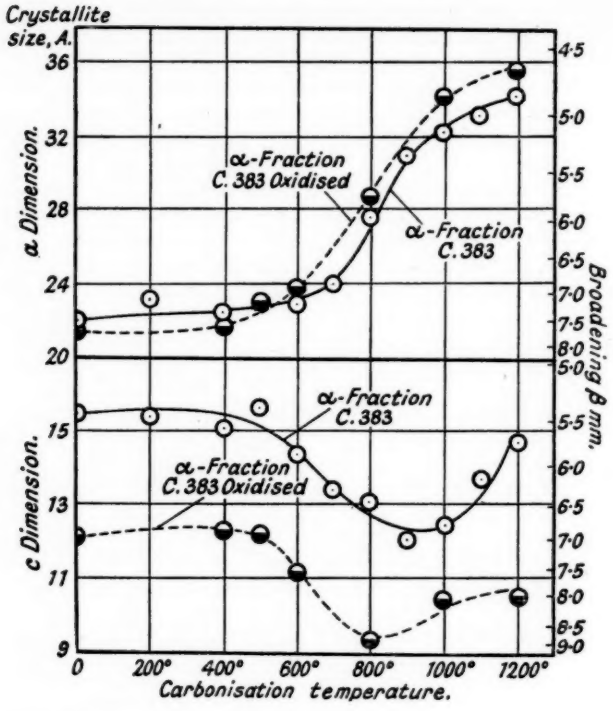

Crystallite size, A.
a Dimension.
c Dimension.
Broadening β mm.
α-Fraction C. 383 Oxidised
α-Fraction C. 383
α-Fraction C. 383
α-Fraction C. 383 Oxidised
Carbonisation temperature.

FIG. 7.

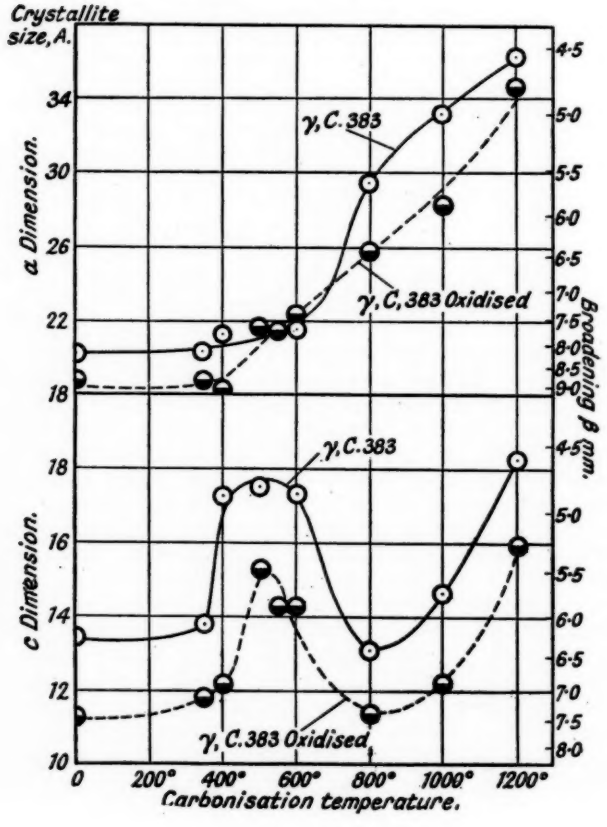
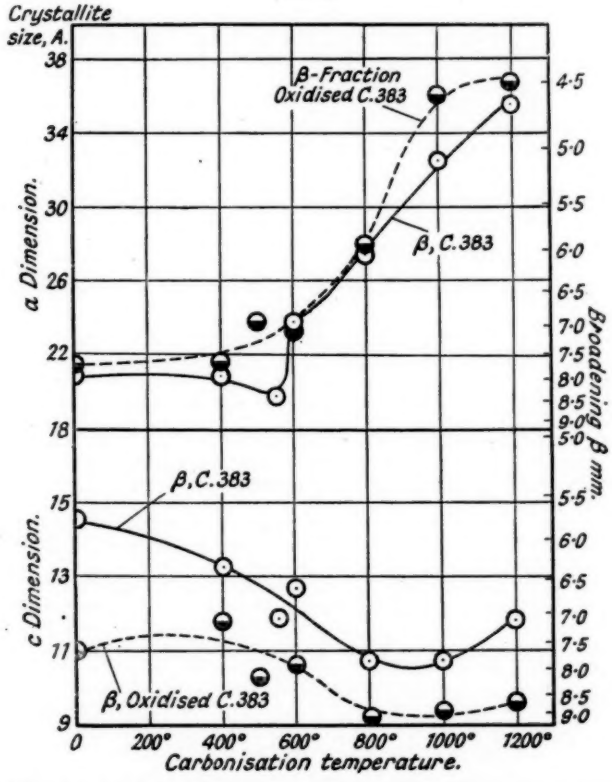

Crystallite size, A.
a Dimension.
c Dimension.
Broadening β mm.
γ, C. 383
γ, C, 383 Oxidised
γ, C. 383
γ, C. 383 Oxidised
Carbonisation temperature.

FIG. 8.

those obtained on the semimicro-scale, probably because of the more complete extraction and smaller losses when using the semimicro-method : it is possible that errors due to the presence of pyridine complexes in the extracts may be greater on the semimicro-scale.

Samples (0·8 g.) of the various fractions were carbonised in a silica boat in an atmosphere of nitrogen at a rate of 5° per min., the final temperature being maintained for 10 mins. The coke yields, which could not be determined accurately, were about 30% from the γ-fraction of the oxidised coal and about 45% from the γ-fraction of the unoxidised coal. *X*-Ray powder photographs of the original γ-extracts and the cokes prepared from them were made by the method already described, and crystallite dimension determinations carried out. The results obtained are given in Figs. 7, 8, and 9. The most interesting feature of these results is that the

FIG. 9.



γ-fraction of the oxidised coal shows a sharp maximum at 500° in its *c*-dimension curve, in spite of the fact that the original oxidised coal shows no caking properties whatsoever. This result, however, in no way invalidates our view that a maximum in the *c*-dimension curve at 500—600° is in some way connected with the phenomenon of caking, for the γ-extract itself, on carbonisation, shows strong caking properties. This result is in accord with the observations of Barash (*Fuel*, 1927, **6**, 532; *J. Soc. Chem. Ind.*, 1929, **48**, 174T, 265T).

The *a*-dimension curves of the various fractions indicate that the oxidation of the coal has had little effect upon the crystallographic behaviour of the fractions on carbonisation, with respect to the *a* dimension. Oxidation, however, brings about important changes in all the fractions, with respect to the *c* dimension. Each of the *c*-dimension curves is depressed some 2—3 A. along its entire length. Experimentally this is a pronounced broadening of the inner 002 diffraction band. The ultimate analyses of the three fractions, unoxidised and oxidised, are shown in Table II.

These results indicate that each fraction had taken up a similar proportion of oxygen. Such systems are, of course, highly complicated and any definite explanation of this crystallographic phenomenon would require further investigation. It is, however, significant that the addition of oxygen to each of the three fractions brings about a decrease in the crystallographic order in

TABLE II.

Composition, % ash-free dry.

	Unoxidised.			Oxidised.		
	C.	H.	Diff.	C.	H.	Diff.
α-Fraction	85·7	4·3	10·0	82·0	3·5	14·5
β-Fraction	84·4	4·8	10·8	80·8	4·8	14·4
γ-Fraction	87·5	6·3	6·2	82·3	7·0	10·7

the cokes and chars prepared from these fractions. This result is in keeping with numerous observations previously reported, concerning the importance of the proportion of oxygen in a carbonaceous substance on its crystallographic behaviour on carbonisation and on the character of the resulting carbon.

The destruction of the caking properties of the original coal by oxidation and the retention of pronounced caking properties in the γ-fraction suggest that the mutual interaction of the fractions and their relative proportions are important factors in determining caking properties.

The above experiments were carried out in the Northern Coke Research Laboratory as part of the fundamental research programme of the British Coke Research Association.

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[*Received, December 16th*, 1947.]

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347. *Some Catalysed Gas-phase Reactions of Aromatic Hydrocarbons. Part III. The Methylation of Naphthalene by Means of Dimethyl Ether.*

By G. P. ARMSTRONG, (MISS) D. H. GROVE, D. LL. HAMMICK, and H. W. THOMPSON.

Naphthalene has been methylated by the action of dimethyl ether on the hydrocarbon in the presence of bauxite. Flow methods at 450° gave yields of about 2% of monomethylnaphthalenes; at about 200—300° and 50—70 atmospheres in an autoclave, yields of monomethylnaphthalenes of about 7% were obtained, from a product containing 30% of methylated naphthalene. The ratio of 1-methyl- to 2-methyl-naphthalene was about 4 : 1.

Pure 1-methylnaphthalene passed over bauxite at 450° gave a mixture (*ca.* 2 : 1) of 1-methyl- and 2-methyl-naphthalene.

Extinction coefficients for naphthalene and the two monomethylnaphthalenes were determined at three wave-lengths in the ultra-violet and used for the identification and determination of methylated products.

IT has been shown in Part I (*J.*, 1947, 928) that at 450° in the presence of catalysts of aluminium silicate type dimethyl ether interacts with benzene in accordance with the scheme:

$$C_6H_6 + (CH_3)_2O = CH_3{\cdot}C_6H_5 + CH_3{\cdot}OH$$
$$2CH_3{\cdot}OH = (CH_3)_2O + H_2O$$

It was pointed out that the small quantities of toluene obtained when methyl alcohol is substituted for dimethyl ether might have been derived from the interaction of the latter which is formed extremely rapidly from methyl alcohol in the presence of the type of catalyst employed. It was in any case to be expected that naphthalene could be methylated by dimethyl ether under similar conditions, and this has in fact proved to be the case.

Naphthalene vapour and dimethyl ether, together with a carrier gas (nitrogen), were led over bauxite at 450° with a contact time of 15—30 seconds. The condensates from a number of runs were collected, and the methylated products separated by fractional distillation. A fraction [sample F(2)β] containing 70% of monomethylnaphthalenes was obtained, corresponding to a minimum yield of 2—3% on the naphthalene. The presence of both monomethyl isomers was established from their ultra-violet absorption spectra, the ratio of 1-methyl to 2-methyl being found to be very nearly 2 : 1. Analysis of the highest-boiling fractions and their picrates indicated the presence of polymethylated naphthalenes.

Interaction of dimethyl ether and naphthalene in an autoclave at 250—300° in the presence of bauxite at pressures between 50 and 70 atmospheres gave much better yields of methylated product; estimated yields of mono- and di-methylnaphthalenes (in a representative run) were 7·5% and 3·5% respectively out of a total yield of 30% of methylated product.

In one run optically pure 1-methylnaphthalene was passed over the bauxite catalyst; the

product was shown spectroscopically to contain 2-methylnaphthalene, the ratio of the isomers being about 4 : 1 of 1-methyl to 2-methyl.

Experimental.

Materials.—Dimethyl ether. The ether was prepared by catalytic dehydrogenation of methyl alcohol over bauxite at 375°. Water and unchanged alcohol were condensed, and the ether absorbed in concentrated sulphuric acid, from which it was liberated by running the solution into water. The ether was passed successively through a double-surface condenser, an ice-cooled trap, and a column of anhydrous calcium chloride. It was finally condensed in an alcohol–solid carbon dioxide freezing mixture and stored in an iron cylinder; b. p. − 26° to − 27°.

Naphthalene. This was twice sublimed and recrystallised from ethyl alcohol, m. p. 80·5°.

1-*Methylnaphthalene.* A sample supplied as "pure" boiled at 233—234°/756 mm. (corr.). It was twice redistilled, and recrystallised by cooling a solution in ethyl alcohol in an alcohol–solid carbon dioxide freezing mixture to about − 70°. The crystals that separated were sucked into and pressed free from liquid on a sintered glass filter immersed in the solution. The solid was removed and allowed to melt. After two recrystallisations the liquid 1-methylnaphthalene was shaken with successive amounts of water, calcium chloride solution, and anhydrous calcium chloride to remove any remaining alcohol. The product was finally distilled [b. p. 242·5—243·0/763 mm. (corr.)]; its ultra-violet absorption spectrum showed no sign of naphthalene or of 2-methylnaphthalene.

2-*Methylnaphthalene.* A "pure" sample was recrystallised thrice from alcohol (to constant m. p.); m. p. 34·4° (corr.).

The monomethylnaphthalenes were required pure as standards in the spectroscopic detection and determination of the isomers in the products of methylation.

Catalyst. The catalyst used was a specimen of bauxite, ground and sieved to 2/5 mesh. Before use it was heated to 500° in a stream of nitrogen for 5 hours (Found: SiO_2, 30·9; Al_2O_3, 35·6; Fe_2O_3, 6·9; TiO_2; 4·3; loss on ignition, 22·7%).

After each run (1—2 hours) the catalyst was always found to be "carbonised"; this is almost certainly due to decomposition of dimethyl ether, as naphthalene could be passed over the catalyst at 450° and recovered practically unchanged in m. p. The carbonised catalyst was reactivated by heating it in a current of oxygen.

Apparatus.—A horizontal "Pyrex" reaction tube was used, 35 mm. in diameter and 85 cm. long, the average charge of catalyst being 450 g. Free space was about 28 c.c. as measured by water displacement. The tube was heated electrically, temperature being measured by means of thermocouples. The nitrogen and dimethyl ether were supplied from cylinders, and their rate of flow measured by flowmeters. The naphthalene was vapourised electrically from a tared "Pyrex" test tube, the mixed vapours being preheated to 300° before entering the catalyst tube, which was kept at *ca.* 450—460°. Molecular ratios of ether to naphthalene lay between 1 : 1 and 1 : 2. The crude product was separated from water, broken up, and dried over concentrated sulphuric acid. Experiments were also carried out under pressure in a gas-heated steel autoclave at 250—300° and about 50—60 atmospheres, under which conditions the reagents were partially liquid.

Separation and Analysis of Products.—Owing to the material collected from both flow and autoclave experiments being solid and the yields of methylated products being comparatively small, rapid analysis was not possible. For this reason no attempt was made in the present series of experiments to vary temperatures, rates, and reagent ratios in a search for optimum conditions; conditions were chosen as nearly analogous as possible to those found most suitable in the methylation of benzene (Given and Hammick, *loc. cit.*). The crude products from 10 runs in the flow apparatus and from the autoclave melted 4—5° below the m. p. of naphthalene, which was removed by distillation through a fractionating column * delivering into a wide upright condenser and receiver.

As an example of the method of separation and determination we quote the following results obtained with the autoclave:

Catalyst (bauxite).	Naphthalene. (*x*)	Dimethyl ether. (*y*)	Mol. ratio. (*x* : *y*)	Temp.	Press.	Time.
100 G.	120 G.	50 G.	1 : 1·2	270—300°	53 atm.	4·5 hrs.

116 G. of oily solid product were recovered, and some naphthalene removed by ordinary distillation. The residue was fractionated and 13 fractions collected between 228° and 315°, totalling 44·2 g.; residual tar weighed 8·9 g. The lower fractions were combined in batches and refractionated; a further 17 g. boiling below 230° were rejected from these fractions. The following fractions were obtained and analysed:

			Found, %.		Calc, %.		
Fraction.	B. p.	Wt., g.	C.	H.	C.	H.	
I	235—242°	4·7	—	—	—	—	
II	244—249	6·0	93·01	7·29	—	—	
III	250—255	2·0	92·76	7·28	92·9 (monomethyl)	7·1	(1)
IV	257—262	2·6	92·3	7·92	92·3	7·7	(2)
V	262—266	0·6			(dimethyl)		
VI	269—278	1·0	91·5	7·99	91·77	8·23	(3)
VII	279—280	1·4			(trimethyl)		

* The packing of the column consisted of a glass rod carrying discs, with lips round the edges to prevent hold-up of the refluxing liquid. The column was heavily lagged with asbestos and was heated by a coil wound in the the outer layers. Flow of current was adjusted in the coil during distillation so as to keep the column temperature 20° below the boiling point of the fraction to be distilled.

Total weight of methylated product was about 36 g., representing 31% on the initial charge of naphthalene, minimum yields of methylnaphthalenes being: monomethylnaphthalenes, 7·5%, dimethylnaphthalenes, 3·5%, trimethylnaphthalenes, 2·0%. From the high-boiling residue a picrate, m. p. 128—130°, was obtained, corresponding to a methylated naphthalene between penta- and hexa-methyl [Found: C, 59·46; H, 5·07. Calc. for $C_{21}H_{21}O_7N_3$ (pentamethyl): C, 59·0; H, 4·92. Calc. for $C_{22}H_{23}O_7N_3$ (hexamethyl): C, 59·9; H, 5·22%].

Spectroscopic Examination and Analysis.—In order to analyse the products, and in particular the fraction containing monomethylnaphthalenes, differences in the ultra-violet absorption spectra of naphthalene, and 1- and 2-methylnaphthalenes, were used. The spectra of these substances have been measured previously, the most relevant determinations being those of Morton and de Gouveia (*J.*, 1934, 916), using solutions in hexane. Since there was some divergence in the measured extinction coefficients, probably because of differences in instrumental resolving power, these were redetermined using a Spekker photometer with Hilger E 315 spectrograph. Since for each of the substances the extinction coefficient alters very steeply with wave-length, it was necessary to use a series of concentrations in hexane in each case.

The absorption of these substances extends from about 3200 A. towards shorter wave-lengths. The bands in the region 3200—2950 A. are the most characteristic and suitable for analysis of mixtures since at shorter wave-lengths there are broader and severely overlapping bands. The most convenient characteristic bands are as follows: naphthalene 3110 A., 1-methylnaphthalene 3140 A., 2-methylnaphthalene 3190 A. The extinction coefficients at each of these wave-lengths were determined for the pure substances with the following results.

	3190 A.	3140 A.	3110 A.
Naphthalene	18	60	260
1-Methylnaphthalene.....	75	330	230
2-Methylnaphthalene.....	540	175	260

Determination of the optical densities of a mixture at each of the 3 wave-lengths, followed by solution of the 3 linear equations, will then give the required analysis. It was known, however, that the samples to be analysed would contain only small percentages of naphthalene, and since high accuracy was not required a simpler method was devised for the analysis. A curve was constructed showing the ratio of optical densities at 3190/3140 A. as a function of percentage of 2-methylnaphthalene in a mixture with the 1-methyl isomer. For pure 1-methylnaphthalene this ratio is 0·23, and for pure 2-methylnaphthalene it is 3·09. A series of test mixtures of different composition showed that this calibration chart was satisfactory. The total amounts of the individual isomers could then be calculated from the optical density at either wave-length. Inexact agreement implies the possible presence of other compounds.

The dimethylnaphthalenes have absorption bands which lie close to those of the monomethyl derivatives, and if much of these were present the whole analysis would be invalid. It was known, however, from the fractionation already described that this would not occur.

The results of 3 typical analyses are given.

I. Sample G (1)α; collected after passage of 1-methylnaphthalene over the catalyst at 450°. This was found to contain 1- and 2-methyl isomers and no detectable naphthalene or other methylated product. The ratio of optical densities at 3190/3140 was 0·575, which corresponds to about 20% of 2-methylnaphthalene.

II. Sample F(2)α; concentrate from a number of flow reactions. The ratio of optical densities was 0·76, corresponding to 30% of 2-methylnaphthalene; only about 70% of the sample would be attributed to monomethylnaphthalenes.

III. Sample F(2)β; similar concentrate to F(2)α. This contained about 30% of the 2-isomer, but the optical densities at a series of wave-lengths suggested that small amounts of the dimethylnaphthalenes were also present.

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348. *Cinnolines. Part XIX. The Preparation and Reactions of Some 8-Nitro-4-hydroxy- and 4-Hydroxy-Bz-methyl-cinnolines.*

By J. R. KENEFORD, J. S. MORLEY, and J. C. E. SIMPSON.

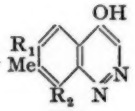
A number of 4-substituted-8-nitro- and -7- and -8-methyl-cinnolines have been synthesised, starting from 2-nitro-5-amino-4-methylacetophenone and 2-nitro-*m*-toluic acid.

In the course of these preparations some further examples of unusual group interchange reactions have been encountered; 3-nitro-2-aminoacetophenone yields, according to conditions, either 8-nitro- or 8-chloro-4-hydroxycinnoline [cf. Parts X and XVIII of this series (*J.*, 1947, 232; this vol., p. 1170)], and 7-chloro-6-nitro-4-hydroxycinnoline gives 4 : 6 : 7-trichlorocinnoline when heated with a mixture of phosphoryl chloride and phosphorus pentachloride.

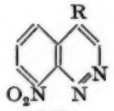
DEAMINATION of 2-nitro-5-amino-4-methylacetophenone (I; R = NH_2), previously prepared from *p*-methylacetophenone (Keneford and Simpson, *J.*, 1947, 227), leads smoothly to 2-nitro-4-methylacetophenone (I; R = H). Reduction of this nitro-ketone gave 2-*amino*-4-

methylacetophenone, which, under the conditions appropriate to this type of amine (Keneford and Simpson, *J.*, 1947,917; Schofield and Simpson, this vol., p. 1170), yielded 4-*hydroxy*-7-*methylcinnoline* (II; $R_1 = R_2 = H$), and thence, by routine methods, 4-*acetoxy*- and 4-*phenoxy*-7-*methylcinnoline*. Nitration of (II; $R_1 = R_2 = H$) yielded a single mono-nitro-derivative, which is regarded as 8-*nitro*-4-*hydroxy*-7-*methylcinnoline* (II; $R_1 = H$; $R_2 = NO_2$). No formal proof of the structure of this substance is available, but the 8-position is, clearly, the likely point of attack; furthermore, 8-nitro-4-hydroxycinnoline (III; R = OH) is a minor product of the nitration of 4-hydroxycinnoline (Simpson, *J.*, 1947, 237); and, finally, 8-nitro-4-hydroxycinnolines show certain properties, such as resistance to acetylation (these properties will be discussed in a future communication), which are exhibited by the nitro-hydroxy-methylcinnoline in question. By the usual procedures, 8-nitro-4-hydroxy-7-methylcinnoline was converted into 4-*chloro*-8-*nitro*-, 8-*nitro*-4-*amino*-, 8-*nitro*-4-*anilino*-, and 8-*nitro*-4-*phenoxy*-7-*methylcinnoline*.

(I.)



(II.)

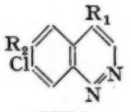


(III.)

(IV.)

(V.)

(VI.)



(VII.)

Nitration of 2-*acetamido*-4-*methylacetophenone* (IV; R = H) gave an almost quantitative yield of a *mononitro*-derivative, m. p. 136—137°, which by hydrolysis and cyclisation was converted into a nitro-hydroxycinnoline, m. p. 250—251°, which was not identical with 8-nitro-4-hydroxy-7-methylcinnoline. The substances, m. p. 250—251° and 136—137°, are therefore regarded as 6-*nitro*-4-*hydroxy*-7-*methylcinnoline* (II; $R_1 = NO_2$; $R_2 = H$) and 5-*nitro*-2-*acetamido*-4-*methylacetophenone* (IV; $R = NO_2$) respectively. The formation of the latter was accompanied by that of a small amount of a dinitro-derivative, evidently 3 : 5-*dinitro*-2-*acetamido*-4-*methylacetophenone*; this was hydrolysed to 3 : 5-*dinitro*-2-*amino*-4-*methylacetophenone*, which, however, did not give a cinnoline derivative under the usual conditions.

Condensation of the chloride of 2-nitro-*m*-toluic acid (Tomisek, Graham, Griffith, Pease, and Christensen, *J. Amer. Chem. Soc.*, 1946, **68**, 1587) with ethyl ethoxymagnesiomalonate (cf. Walker and Hauser, *J. Amer. Chem. Soc.*, 1946, **68**, 1386) proceeded smoothly, and yielded, after hydrolysis, 2-*nitro*-3-*methylacetophenone* (V; $R = NO_2$). This was reduced to 2-*amino*-3-*methylacetophenone* (V; $R = NH_2$), from which 4-*hydroxy*-8-*methylcinnoline* (VI; R = Me) and thence, by the usual methods, 4-*phenoxy*-, 4-*amino*-, and 4-*anilino*-8-*methylcinnoline*, were prepared. Nitration of (VI; R = Me) gave in high yield a mono-nitro-derivative; this is probably 5- or 7-*nitro*-4-*hydroxy*-8-*methylcinnoline*. From the nitro-compound, 5- or 7-*nitro*-4-*phenoxy*-, -4-*amino*-, and -4-*anilino*-8-*methylcinnoline* were prepared by known methods.

Conversion of 8-nitro-4-hydroxycinnoline (III; R = OH) (Simpson, *loc. cit.*) into the 4-chloro-compound and treatment of the latter with phenol and ammonium carbonate gave both 8-*nitro*-4-*phenoxy*- (III; R = OPh) and 8-*nitro*-4-*amino*-*cinnoline* (III; $R = NH_2$). This is the first example in the cinnoline series in which this method of phenoxylation produces an appreciable amount of the corresponding amino-compound, although both reactions occur with 4-chloro-6- and -7-nitroquinazoline (Morley and Simpson, this vol., p. 360), and the amine is usually the sole product in the case of 5-chloroacridines (*e.g.*, Albert and Gledhill, *J. Soc. Chem. Ind.*, 1945, **64**, 169).

As an alternative route to (III; $R = NH_2$), the nitration of 4-chlorocinnoline was investigated, as it has been established that 4-chloro-8-nitroquinoline is a major product of the nitration of 4-chloroquinoline (cf., *e.g.*, Gouley, Moersch, and Mosher, *J. Amer. Chem. Soc.*, 1947, **69**, 303; Simpson and Wright, following paper). A crude nitration product, formed in poor yield, gave 8-nitro-4-phenoxycinnoline on phenoxylation, but the reaction was not of practical value, as 4-chlorocinnoline is considerably more resistant to nitration than 4-chloroquinoline, and side-reactions occur if the conditions are made sufficiently drastic to ensure that more than a small fraction of the chloro-compound is nitrated.

During this work it was observed that if 3-nitro-2-aminoacetophenone is diazotised and cyclised in concentrated hydrochloric acid, the product is not (III; R = OH) (obtained in sulphuric acid) but 8-chloro-4-hydroxycinnoline (VI; R = Cl) (Schofield and Simpson, *J.*, 1945, 520). The precise stage at which the exchange of groups occurs was not ascertained, but the reaction is probably a further example of the type of group-exchange, discussed in Parts X and XVIII (*J.*, 1947, 232; this vol., p. 1170), which is undergone by diazonium kations derived from certain *o*-aminoacetophenones before cyclisation. Group interchange can, however, also occur in a preformed cinnoline nucleus; thus, as examples involving a substituent in the *Bz*-ring, Leonard and Boyd (*J. Org. Chem.*, 1946, **11**, 419) have found that 6-bromo-4-hydroxycinnoline yields what is undoubtedly 4 : 6-dichlorocinnoline (cf. Keneford and Simpson, *J.*, 1947, 917) when treated with a mixture of phosphorus pentachloride and oxychloride at 135—140°, and we have now found that 7-chloro-6-nitro-4-hydroxycinnoline (VII; R_1 = OH; R_2 = NO_2) (Atkinson and Simpson, *J.*, 1947, 232) similarly yields 4 : 6 : 7-trichlorocinnoline (VII; R_1 = R_2 = Cl) instead of the expected 4 : 7-dichloro-6-nitrocinnoline; and Dr. K. Schofield informs us (private communication) that group-exchange may also occur in the azine ring of the cinnoline nucleus, 3-bromo-4-hydroxycinnolines giving, under suitable conditions, the 3 : 4-dichloro-compounds.

Experimental.

M. p.s are uncorrected. Nitrations were performed with mechanical stirring. Unless otherwise stated, 4-chloro- and 4-phenoxy-cinnolines were prepared by the methods previously described (Keneford and Simpson, *J.*, 1947, 917) (phenoxy-compounds were better isolated by direct filtration than by ether-extraction). 4-Amino-compounds were prepared from the phenoxy-compounds and fused ammonium acetate by the method of Keneford, Schofield, and Simpson (this vol., p. 358); optimum conditions varied in different cases as detailed below.

2-Amino-4-methylacetophenone.—A hot solution of 2-nitro-5-amino-4-methylacetophenone (24 g.) in concentrated hydrochloric acid (760 c.c.) was rapidly cooled, and the resultant suspension diazotised at 0—5° with a solution of sodium nitrite (10 g.) in water (30 c.c.); cold hypophosphorous acid (30%, 280 c.c., 10 moles) was added with stirring during ½—1 hour, and the mixture left at 0° for 1 week. The product was collected with ether, the solution washed (aqueous sodium hydroxide and water), dried, solvent removed, and the crude 2-nitro-4-methylacetophenone (83% yield) distilled at 120—130°/1 mm. (yield of purified ketone, 75%). The use of 15 and 5 moles of hypophosphorous acid gave crude yields of 85% and 73% respectively. Reduction of the nitro-ketone (49·6 g.) with iron powder (100 g.), water (200 c.c.), and acetic acid (200 c.c.) by the method previously described (*J.*, 1945, 646) gave *2-amino-4-methylacetophenone* (crude yield, 41·5 g., m. p. 50—52°), which crystallised from ligroin (b. p. 40—60°) in brittle amber blades, m. p. 55—56° (Found: C, 72·2; H, 7·45. $C_9H_{11}ON$ requires C, 72·45; H, 7·45%), and yielded (with acetic anhydride at 95°) *2-acetamido-4-methylacetophenone* (95%) as long prismatic needles, m. p. 75—76°, from ligroin (b. p. 60—80°) (Found: C, 68·35; H, 6·85; N, 7·5. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·85; N, 7·35%).

5-Nitro-2-amino-4-methylacetophenone.—2-Acetamido-4-methylacetophenone (5 g.) was added during 25 minutes to a mixture of nitric acid (*d* 1·483) and concentrated sulphuric acid (5 : 2 v/v, 30 c.c.) at − 8° to 0°. After a further 10 minutes, the solution was poured on ice; *5-nitro-2-acetamido-4-methylacetophenone*, precipitated in almost quantitative yield, separated from alcohol in long, cream-coloured needles, m. p. 136—137° (Found: C, 56·05; H, 4·95; N, 11·65. $C_{11}H_{12}O_4N_2$ requires C, 55·9; H, 5·15; N, 11·85%). Hydrolysis with 5N-hydrochloric acid (10 parts by vol., ½ hour's reflux) gave, quantitatively, *5-nitro-2-amino-4-methylacetophenone*, which formed long greenish-yellow prismatic needles, m. p. 165·5—166·5°, from alcohol (Found: N, 14·25. $C_9H_{10}O_3N_2$ requires N, 14·45%).

3 : 5-Dinitro-2-amino-4-methylacetophenone.—The acid filtrate from the above acetamido-compound was basified (sodium carbonate) and extracted with ether, the extract dried and concentrated, and the solid residue (0·2 g.) recrystallised from benzene–ligroin (b. p. 60—80°), yielding *3 : 5-dinitro-2-acetamido-4-methylacetophenone* as lemon-yellow elongated polyhedra, m. p. 183—183·5° (Found: C, 47·1; H, 4·05; N, 15·2. $C_{11}H_{11}O_6N_3$ requires C, 46·95; H, 3·95; N, 14·95%). Hydrolysis of this as above gave in good yield *3 : 5-dinitro-2-amino-4-methylacetophenone*, deep yellow micro-prisms, m. p. 192—193°, from alcohol (Found: N, 17·7. $C_9H_9O_5N_3$ requires N, 17·55%), which gave no crystalline product when its solution in acetic acid–85% sulphuric acid was diazotised and heated.

2-Nitro-3-methylacetophenone.—2-Nitro-*m*-toluic acid (72·4 g.), phosphorus pentachloride (91·7 g.), and benzene (100 c.c.) were refluxed for ¾ hour, solvent removed, and the residue again evaporated with benzene under reduced pressure. A solution of the residue in ether–benzene (1 : 1, 400 c.c.) was added with stirring, during ½ hour at room temperature, to ethyl ethoxymagnesiomalonate [from magnesium (10·7 g.), alcohol (10 c.c.), carbon tetrachloride (1 c.c.), ether (150 c.c.), and a solution of ethyl malonate (70·4 g.) in alcohol (40 c.c.) and ether (50 c.c.)], and the suspension was refluxed for ½ hour and acidified with dilute sulphuric acid. The organic product was dried and freed from solvent, and the residue, which crystallised overnight, was refluxed for 5 hours with a mixture of acetic acid (120 c.c.), concentrated sulphuric acid (15 c.c.), and water (80 c.c.). Basification with 20% aqueous sodium hydroxide at 0° and extraction with ether gave *2-nitro-3-methylacetophenone* (57·9 g., 81% based on 2-nitro-*m*-toluic acid), which formed colourless brittle rods, m. p. 85—86°, from ligroin (b. p. 60—80°) (Found: C, 60·45; H, 5·05; N, 7·9. $C_9H_9O_3N$ requires C, 60·3; H, 5·05; N, 7·8%).

2-Amino-3-methylacetophenone.—Reduction of the nitro-ketone (45 g.) with iron powder (90 g.), acetic acid (225 c.c.), and water (225 c.c.) at 95° gave *2-amino-3-methylacetophenone* (36·7 g., 98%), which crystallised from ligroin (b. p. 60—80°) in pale amber, brittle plates, m. p. 55—56° (Found: C, 72·9; H, 7·7. $C_9H_{11}ON$ requires C, 72·4; H, 7·4%). *2-Acetamido-3-methylacetophenone* (prepared with acetic

anhydride at 95°) formed colourless rhombohedra, m. p. 145—146°, from alcohol (Found: N, 7·45. $C_{11}H_{13}O_2N$ requires N, 7·3%).

4-Hydroxy-7-methylcinnoline.—A hot solution of 2-amino-4-methylacetophenone (33·6 g., m. p. 50—52°) in concentrated hydrochloric acid (850 c.c.) was quickly cooled, diazotised (16·7 g. of sodium nitrite in 24 c.c. of water), filtered, treated with concentrated hydrochloric acid (2550 c.c.), kept at 50—60° for 2 hours, and then concentrated under reduced pressure to a small volume. Addition of saturated aqueous sodium acetate precipitated *4-hydroxy-7-methylcinnoline* (21 g., m. p. 235—237°); this was very soluble in alcohol, and crystallised from acetic acid in colourless silky needles, m. p. 243·5—244·5° (Found: C, 67·35; H, 4·95; N, 17·85. $C_9H_8ON_2$ requires C, 67·45; H, 5·05; N, 17·5%). *4-Acetoxy-7-methylcinnoline*, prepared by refluxing the hydroxy-compound with acetic anhydride (6 parts) for ½ hour, separated from alcohol in tufts of jagged needles, m. p. 117—118° (Found: C, 65·2; H, 4·75; N, 14·0. $C_{11}H_{10}O_2N_2$ requires C, 65·3; H, 5·0; N, 13·85%).

4-Phenoxy-7-methylcinnoline.—The hydroxycinnoline (1 g.), phosphorus pentachloride (2·5 g.), and phosphorus oxychloride (3 c.c.) reacted spontaneously, and no further change seemed to occur on heating. The crude chloro-compound (bright yellow glittering scales, m. p. 115—117°) (0·85 g.) gave *4-phenoxy-7-methylcinnoline* (0·9 g.), which formed colourless prismatic needles, m. p. 113—114°, from benzene–ligroin (b. p. 60—80°) (Found: C, 76·1; H, 5·3. $C_{15}H_{12}ON_2$ requires C, 76·25; H, 5·15%).

8-Nitro-4-hydroxy-7-methylcinnoline.—4-Hydroxy-7-methylcinnoline (10 g.) was added during ½ hour to a mixture of nitric acid (*d* 1·53) and 65% oleum (5 : 2 v/v, 60 c.c.) at 0—5°. After a further ½ hour at 0°, the solution was poured on ice (500 g.) and the crude solid [11·85 g., m. p. 228—232° (decomp.)] collected. Digestion with hot alcohol and crystallisation from acetic acid gave lemon-yellow jagged blades, m. p. 243—244° (decomp.), of *8-nitro-4-hydroxy-7-methylcinnoline* (Found: C, 52·7; H, 3·6; N, 20·4. $C_9H_7O_3N_3$ requires C, 52·65; H, 3·45; N, 20·45%), unchanged after being refluxed with acetic anhydride for 4 hours.

4-Chloro-8-nitro-7-methylcinnoline.—The crude chloro-compound [5·2 g., m. p. 195° (decomp.)] prepared from the above substance (5 g.), phosphorus pentachloride (12·5 g.), and phosphorus oxychloride (15 c.c.) was collected by filtration of the alkaline suspension; crystallisation from benzene gave pale green blades of *4-chloro-8-nitro-7-methylcinnoline*, m. p. 210—211° (decomp.), sparingly soluble in ether (Found: C, 48·65; H, 3·0; Cl, 16·5. $C_9H_6O_2N_3Cl$ requires C, 48·3; H, 2·7; Cl, 15·9%). The optimum reaction time is 5—10 minutes; with 1 hour's heating, much dark, insoluble matter is formed, and only 25—30% of impure chloro-compound is isolable by extraction with a large volume of ether.

8-Nitro-4-anilino-7-methylcinnoline.—A mixture of aniline (0·1 g.), the foregoing chloro-compound (0·25 g.), 50% aqueous acetone (110 c.c.), and concentrated hydrochloric acid (6 drops) was refluxed for ½ hour. The solid (0·15 g.) obtained by basification with ammonia was recrystallised from alcohol, from which *8-nitro-4-anilino-7-methylcinnoline* separated in greenish-yellow prismatic needles, m. p. 262—263° (decomp.) (Found: N, 19·7. $C_{15}H_{12}O_2N_4$ requires N, 20·0%). Dilution of the ammoniacal filtrate with water gave unchanged chloro-compound.

8-Nitro-4-phenoxy-7-methylcinnoline.—Reaction was incomplete at 95°, but at 150° (bath) 4-chloro-8-nitro-7-methylcinnoline gave *8-nitro-4-phenoxy-7-methylcinnoline* in 84% yield as cream-coloured prismatic needles, m. p. 172—173°, from alcohol (Found: C, 64·05; H, 4·25. $C_{15}H_{11}O_3N_3$ requires C, 64·05; H, 3·95%).

8-Nitro-4-amino-7-methylcinnoline.—The reaction temperature, initially 160—170°, was raised to 200° and kept there for ½ hour (air-condenser). Dilution with water gave a solid [3·2 g., m. p. 255—260° (decomp.), from 4·1 g. of phenoxy-compound] which was extracted with 20% aqueous acetic acid; the insoluble fraction (1·1 g.) was unchanged phenoxy-compound, and the filtrate on basification (ammonia) yielded *8-nitro-4-amino-7-methylcinnoline* (1·92 g.), which, after crystallisation from alcohol, formed sandy micro-prisms, m. p. 300° (decomp.) (Found: C, 53·35; H, 4·05; N, 27·8. $C_9H_8O_2N_4$ requires C, 52·9; H, 3·95; N, 27·45%).

6-Nitro-4-hydroxy-7-methylcinnoline.—A hot solution of 5-nitro-2-amino-4-methylacetophenone (1 g.) in concentrated hydrochloric acid (50 c.c.) was quickly cooled, diazotised [sodium nitrite (0·4 g.) in water (5 c.c.)], heated at 90° for ½ hour, and poured into water. Crystallisation of the crude product (0·8 g., 76%, m. p. 244—246°) from acetic acid yielded *6-nitro-4-hydroxy-7-methylcinnoline* as pale brown rhombohedra, m. p. 250—251° (200—210° when mixed with the 8-nitro-isomer) (Found: C, 52·9; H, 3·15. $C_9H_7O_3N_3$ requires C, 52·65; H, 3·45%).

8-Chloro-4-hydroxycinnoline from 3-Nitro-2-aminoacetophenone.—A filtered solution, prepared as in similar cases (above) from 3-nitro-2-aminoacetophenone (2 g.), concentrated hydrochloric acid (110 c.c.), and sodium nitrite (0·85 g.) in water (1·5 c.c.), was kept at 70—80° for ½ hour, concentrated (reduced pressure) to a small volume, and treated with sodium acetate. The solid so obtained (0·9 g., 45%, m. p. 192—194°) separated from alcohol in soft greenish-yellow needles, m. p. 195—196°, not depressed by admixture with authentic 8-chloro-4-hydroxycinnoline.

8-Chloro-4-phenoxycinnoline.—The crude chloro-compound from the above substance (0·65 g.), phosphorus pentachloride (1·3 g.), and phosphorus oxychloride (1·8 c.c.) formed yellow spikes, m. p. 146—147° (0·6 g.), from ether, and yielded *8-chloro-4-phenoxycinnoline* (0·45 g. from 0·5 g.) as colourless prismatic needles, m. p. 158—159°, from benzene–ligroin (b. p. 60—80°) (Found: C, 64·8; H, 3·7. $C_{14}H_9ON_2Cl$ requires C, 65·5; H, 3·55%).

8-Nitro-4-phenoxy- and -4-amino-cinnoline.—(*a*) The method previously described (*J.*, 1947, 237) for the preparation of 8-nitro-4-hydroxycinnoline gave a somewhat improved yield on a larger scale, 7·5 g. (70%) resulting from 10·1 g. of 3-nitro-2-aminoacetophenone. 4-Chloro-8-nitrocinnoline (2·8 g. from 3 g. of hydroxy-compound) formed yellow needles, m. p. 180° (decomp.), from benzene. Treatment of this (1 part) with phenol (7·5 parts) and powdered ammonium carbonate (5 parts) at 90—95° for 1 hour gave, by basification and ether-extraction, a crude product which was digested with 20% aqueous acetic acid. The insoluble fraction (from 4·8 g. of chloro-compound), after recrystallisation from benzene, yielded *8-nitro-4-phenoxycinnoline* (0·8 g., 13%) as pale green prismatic needles, m. p. 166—167° (Found: C, 63·05; H, 3·2; N, 15·6. $C_{14}H_9O_3N_3$ requires C, 62·9; H, 3·4; N, 15·7%). The acid-soluble material was *8-nitro-4-aminocinnoline* (2·17 g., 50%); this base had a marked solubility lag in alcohol, from which

it separated in minute rust-red prismatic needles, m. p. 242—243° (decomp.) (Found : C, 47·1; H, 3·75; N, 28·2. $C_8H_6O_2N_4,\frac{3}{4}H_2O$ requires C, 47·15; H, 3·75; N, 27·5. $C_8H_6O_2N_4,\frac{3}{2}H_2O$ requires C, 47·5; H, 3·65; N, 27·7%).

(*b*) 4-Chlorocinnoline (5 g.) was added during ½ hour to a mixture of nitric acid (*d* 1·53) and 65% oleum (5 : 2 v/v, 27 c.c.) kept at 40—45°. After a further hour at this temperature, the red solution was poured on ice; the mixture was made just alkaline (much alkali-soluble matter was present) and extracted with ether, the dried extract concentrated, and the solid material (1·45 g., m. p. 145—160°) added to phenol (7 g.) and ammonium carbonate (4 g.). After 1 hour on the steam-bath, the usual isolation procedure yielded 8-nitro-4-phenoxycinnoline (no 8-nitro-4-aminocinnoline was encountered) (0·25 g., 13·5%), m. p. 166—167° alone and mixed with the sample described in (*a*). The use of a shorter reaction time and/or lower temperatures in the nitration gave little or no nitration product, and much 4-chlorocinnoline was recovered.

4-Hydroxy-8-methylcinnoline.—The filtered solution, prepared as in similar cases from 2-amino-3-methylacetophenone (30 g.), concentrated hydrochloric acid (1 l.), sodium nitrite (16·5 g.), and water (25 c.c.), was treated with more concentrated hydrochloric acid (2 l.) and the whole left in the dark at room temperature for 7 days. The product which had separated was combined with that obtained by evaporating the filtrate under reduced pressure, dissolved in aqueous sodium hydroxide, filtered (charcoal), and acidified with acetic acid; *4-hydroxy-8-methylcinnoline* (25·3 g., 78%) separated in fine colourless needles, m. p. 220—221° after crystallisation from aqueous alcohol or aqueous acetic acid (Found : C, 68·0; H, 4·9; N, 17·4. $C_9H_8ON_2$ requires C, 67·5; H, 5·0; N, 17·5%). Cyclisation at 50° reduced the yield to 65%. The compound was unchanged after being refluxed for 3 hours with acetic anhydride.

4-Phenoxy-8-methylcinnoline.—4-Chloro-8-methylcinnoline [from the hydroxy-compound (2 g.), phosphorus pentachloride (3 g.), and phosphorus oxychloride (4 c.c.)] (98% yield; glittering pale-green rods, m. p. 92—93°, from ligroin) had basic properties, could be crystallised unchanged from aqueous ethanol, and was unchanged after standing under aqueous ammonia (*d* 0·88, 5 days at room temperature). Interaction was incomplete when it (1·5 g.) was added with powdered ammonium carbonate (6 g.) to warm phenol (15 g.), the whole being kept at 95° for ¾ hour; the crude product gave *4-phenoxy-8-methylcinnoline* (0·9 g.) as colourless prisms, m. p. 96·5—97°, when crystallised from ligroin (b. p. 40—60°) (Found : C, 76·0; H, 5·0. $C_{15}H_{12}ON_2$ requires C, 76·25; H, 5·1%), and the first ligroin filtrate gave material from which pure chloro-compound (0·32 g.) was isolated.

4-Amino-8-methylcinnoline.—The reaction temperature, initially 130°, was rapidly raised to 180—185° and kept there for 5 minutes. The cold melt was dissolved in water, made just alkaline with ammonia, and filtered (charcoal); the filtrate slowly deposited *4-amino-8-methylcinnoline* (99%), which crystallised from water in almost colourless glittering blades, m. p. 142—147°, 145—152°, or intermediately according to the rate of heating, after softening at 120—130° (Found : C, 64·9; H, 5·9; N, 25·0. $C_9H_9N_3,\frac{1}{2}H_2O$ requires C, 64·3; H, 6·0; N, 25·0%).

4-Anilino-8-methylcinnoline.—4-Chloro-8-methylcinnoline (0·36 g.), aniline (0·22 g.), 50% aqueous acetone (20 c.c.), and concentrated hydrochloric acid (3 drops) were refluxed for ½ hour. Concentration and basification yielded *4-anilino-8-methylcinnoline* (0·46 g.), which formed silky, lemon-yellow needles, m. p. 231—232°, from alcohol (Found : C, 76·2; H, 5·3; N, 18·3. $C_{15}H_{13}N_3$ requires C, 76·6; H, 5·55; N, 17·9%).

5- or 7-Nitro-4-hydroxy-8-methylcinnoline.—4-Hydroxy-8-methylcinnoline (10 g.) was added during ½ hour at — 5° to 0° to a mixture of nitric acid (*d* 1·50) and concentrated sulphuric acid (5 : 2 v/v, 75 c.c.). After a further ½ hour the solution was poured on ice (1 kg.), and the solid collected (11·2 g., 87%, m. p. 251—252°) and recrystallised from aqueous acetic acid, yielding 5- or *7-nitro-4-hydroxy-8-methylcinnoline* as almost colourless needles, m. p. 255—256° after darkening at 245° (Found : C, 52·0; H, 3·1; N, 20·9. $C_9H_7O_3N_3$ requires C, 52·6; H, 3·5; N, 20·5%). The compound was unaffected by 1 hour's refluxing with acetic anhydride.

5- or 7-Nitro-4-phenoxy-8-methylcinnoline.—The hydroxy-compound (10 g.), phosphorus pentachloride (12 g.), and phosphorus oxychloride (40 c.c.) were well mixed and warmed on the water-bath for 3—5 minutes. The mixture was immediately cooled (prolonged heating seriously reduces the yield of product) and diluted with ligroin, and the solid collected, suspended in N/4-sodium hydroxide, and extracted with chloroform. The crude chloro-compound obtained by evaporation of the dried extract formed pale yellow-green prisms, m. p. 130—131° (10·03 g., 93%). It was recovered almost quantitatively after treatment with phenol and powdered ammonium carbonate at 95° for 1 hour. Addition of the chloro-compound (7·5 g.) to a solution of potassium hydroxide (1·9 g.) in phenol (20 g.), followed by heating at 150° (bath) for 1 hour, gave 5- or *7-nitro-4-phenoxy-8-methylcinnoline* (7·66 g., 81%) as almost colourless, glittering rods, m. p. 160—161°, from benzene–ligroin (Found : C, 64·2; H, 4·0; N, 14·6. $C_{15}H_{11}O_3N_3$ requires C, 64·0; H, 3·95; N, 14·95%).

5- or 7-Nitro-4-amino-8-methylcinnoline.—The phenoxy-compound (1 g.) was added to ammonium acetate (10 g.) at 180° (bath), the temperature quickly raised to 195°, the melt immediately cooled and diluted with water, and the solid collected. Digestion with warm 20% acetic acid left a residue (0·42 g.) of almost pure phenoxy-compound, and addition of ammonia precipitated 5- or *7-nitro-4-amino-8-methylcinnoline* (0·24 g.), which formed orange-red prismatic needles, m. p. 242—243° (decomp.), from alcohol (Found : C, 53·3; H, 3·95; N, 26·9. $C_9H_8O_2N_4$ requires C, 52·9; H, 4·0; N, 27·45%).

5- or 7-Nitro-4-anilino-8-methylcinnoline.—Prepared from 4-chloro-5- or 7-nitro-8-methylcinnoline (0·2 g.), aniline (0·1 g.), 50% aqueous acetone (10 c.c.), and concentrated hydrochloric acid (2 drops), 5- or *7-nitro-4-anilino-8-methylcinnoline* (0·24 g.) crystallised from aqueous alcohol in soft, orange-red needles, m. p. 166—168° (Found : C, 63·9; H, 4·3; N, 20·3. $C_{15}H_{12}O_2N_4$ requires C, 64·2; H, 4·3; N, 20·0%).

4 : 6 : 7-Trichlorocinnoline from 7-Chloro-6-nitro-4-hydroxycinnoline.—Treatment of 7-chloro-6-nitro-4-hydroxycinnoline either as in the foregoing cases or with phosphorus oxychloride alone gave a chloro-derivative, m. p. 141—142° (Keneford and Simpson, *J.*, 1947, 227, give m. p. 141·5—142·5° for 4 : 6 : 7-trichlorocinnoline). Identification was established by conversion (with phenol and ammonium

carbonate at 95°) into 6 : 7-dichloro-4-phenoxycinnoline, m. p. 162—163° alone and when mixed with an authentic specimen (Keneford and Simpson, *loc. cit.*).

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349. *Derivatives of 4-Chloro- and 6-Nitro-quinoline.*

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The preparation of some 4-substituted quinoline derivatives, starting from 6-nitro- and 4-chloro-quinoline, is described.

THE following experiments are now recorded in view of the recent appearance of other publications on similar lines.

Bachman and Cooper (*J. Org. Chem.*, 1944, **9**, 302) have described the preparation of chloronitroquinolines from 6-nitroquinoline *N*-oxide and phosphorus oxychloride. The two major products were formulated as 4- and 2-chloro-6-nitroquinoline, but the orientation was not rigidly established, and a third (and minor) product was regarded as 3-chloro-6-nitroquinoline. In repeating this work, we found, in agreement with Bachman and Cooper, that three isomers are formed; we established the constitution of the main product as 4-chloro-6-nitroquinoline by converting it into 6-*nitro*-4-*phenoxyquinoline* and thence into 6-*nitro*-4-*aminoquinoline*, identical with one of the products of the nitration of 4-aminoquinoline (Simpson and Wright, in the press). Reduction of 6-nitro-4-aminoquinoline gave a crystalline, but unstable, diamine; this formed a stable *diacetyl* derivative which reacted readily with methyl iodide and yielded 4 : 6-bisacetamidoquinoline methiodide, from which the corresponding *methochloride* was prepared. 4-Chloro-6-nitroquinoline was also converted by standard methods into 6-*nitro*-4-*anilino*- and 6-*nitro*-4-*methoxy-quinoline*; and 6-*nitro*-2-*phenoxy*- and 6-nitro-2-amino-quinoline (Tschitschibabin, Witkowsky, and Lapschin, *Ber.*, 1925, **58**, 803) were prepared from 2-chloro-6-nitroquinoline.

By the nitration of 4-chloroquinoline we obtained a crude product in high yield, from which about 27% (based on 4-chloroquinoline) of a mono-nitro-derivative, m. p. 127—129°, was readily isolated; this was converted into the corresponding 4-hydroxy-, m. p. 201°, -methoxy-, m. p. 181°, -*phenoxy*-, m. p. 116°, and -amino-derivative, m. p. 230°. After these experiments had been carried out, the publications of Riegel *et al.* (*J. Amer. Chem. Soc.*, 1946, **68**, 1264), Baker *et al.* (*ibid.*, pp. 1267, 1532), and Gouley *et al.* (*ibid.*, 1947, **69**, 303) appeared, in which the same reactions are described and the products are identified as 8-nitro-derivatives. Our attempts to obtain 4-chloro-8-nitroquinoline as the sole product of the nitration of 4-chloroquinoline were unsuccessful; the experience of the American workers was essentially similar to our own, and Gouley *et al.* (*loc. cit.*) isolated 4-chloro-5-nitroquinoline as a second product from this reaction.

The foregoing 4-amino-compounds, and also 4-aminoquinoline, were prepared, like analogous cinnoline (Keneford, Schofield, and Simpson, this vol., p. 358), and quinazoline (Morley and Simpson, *ibid.*, p. 360) derivatives, by fusing the phenoxy-compounds with ammonium acetate.) The three types of heterocyclic amine differ, however, in their behaviour towards alkyl halides. Whereas quaternary salts of 4-aminoquinazolines are too reactive to be isolated (Morley and Simpson, *loc. cit.*), 4-aminoquinolines react smoothly with alcoholic methyl iodide, and thus resemble 4-aminocinnolines (Simpson, *J.*, 1947, 1653, and unpublished work). That the ring nitrogen of the quinoline molecule is attacked was shown by the conversion of 4-aminoquinoline methiodide and 6-*nitro*-4-*aminoquinoline methiodide* into *N*-methyl-4-quinolone and 6-*nitro*-N-*methyl*-4-*quinolone*, respectively, on treatment with hot aqueous alkali hydroxide; the products were authenticated by methylation of the respective hydroxy-compounds with methyl sulphate. 8-*Nitro*-4-*aminoquinoline methiodide* was also prepared; its structure was not proved, but it, too, is doubtless formed by alkylation of the ring nitrogen.

EXPERIMENTAL.

(Melting points are uncorrected.)

6-*Nitroquinoline* N-*Oxide*.—The procedure of Bachman and Cooper (*loc. cit.*) was modified as follows. A solution of 6-nitroquinoline (23·7 g.) in chloroform (150 c.c.) was treated with a chloroform solution (350 c.c.) of perbenzoic acid (5% excess), left at 0° for 48 hours, washed with aqueous ammonia and water,

dried, concentrated, and treated with ether, giving pure *N*-oxide, m. p. 215—217°; average yield, 43·6% [lit. m. p. 220—222° (corr.); yield unspecified].

4-, 3(?)-, *and* 2-*Chloro*-6-*nitroquinoline*.—The foregoing *N*-oxide (30 g.) was refluxed for 1 hour with phosphorus oxychloride (150 c.c.), and the clear solution was then poured on ice (750 g.). After hydrolysis of the oxychloride was complete, the suspension was filtered (filtrate *A*), and the solid (well washed and dried) was recrystallised from chloroform, yielding 2-chloro-6-nitroquinoline as amber-coloured needles, m. p. 224—226° (average yield, 5·6%). Filtrate *A* was basified (ice and ammonia), and the total solid fractionally extracted with cold 2N-hydrochloric acid (150 c.c. portions). The first 2 extracts gave a mixture (7·5 g., m. p. 120—132°) which could not be purified. Further extraction yielded 4-chloro-6-nitroquinoline, which formed colourless needles, m. p. 145—145·5°, after repeated crystallisation from chloroform–ligroin (b. p. 60—80°) (average yield, 33·2%); and finally, using 5N-acid, a substance was isolated which after crystallisation from benzene–ligroin (b. p. 60—80°) formed pale brown needles, m. p. 147—148° (105—110° when mixed with 4-chloro-6-nitroquinoline) (average yield, 2·8%), and was evidently the compound regarded by Bachman and Cooper as 3-chloro-6-nitroquinoline.

Nitration of 4-*Chloroquinoline*.—The crude mixture of 2- and 4-chloroquinoline prepared by the method of Bobranski (*Ber.*, 1938, **71**, 580) was readily separated by extracting its ethereal solution with 0·4N-hydrochloric acid; basification of the acid washings and extraction with ether gave 4-chloroquinoline (50% of mixed chloro-compounds) as an oil, b. p. 128—130°/15—17 mm., which crystallised on standing. This was nitrated as follows: (*a*) A solution of the compound (5 g.) in concentrated sulphuric acid (10 c.c.) was added during 20 minutes to nitric acid (10 c.c., *d* 1·53) at −15° with stirring; on removal of the mixture from the freezing bath, the temperature rose to 36° and then slowly fell. The solution was poured on ice (200 g.), the mixture basified, and the solid collected, dried (5·5 g., m. p. 70—90°), and repeatedly crystallised from alcohol or benzene–ligroin (b. p. 60—80°), yielding prismatic needles or irregular leaflets, respectively, of 4-chloro-8-nitroquinoline, m. p. 127—129° (lit., 126—127°, 129—130°) (average yield, 28% pure) (Found: N, 13·7; Cl, 17·6. Calc. for $C_9H_5O_2N_2Cl$: N, 13·4; Cl, 17·0%). (*b*) The chloro-compound (13·8 g.) was added during 20 minutes to a mixture of oleum (28 c.c., *d* 2·01) and nitric acid (55 c.c., *d* 1·53) at −3° to +5°. After a further 20 minutes the product was worked up as in (*a*) (crude yield, 14 g., m. p. 88—95°; average pure yield, 27%).

6-*Nitro*-4-*methoxyquinoline*.—Prepared from 4-chloro-6-nitroquinoline (0·4 g.), sodium methoxide (0·25 g.), and methanol (10 c.c.) (refluxed for ¾ hour), this *compound* (0·35 g.; 90%) crystallised from benzene–ligroin (b. p. 60—80°) in fine colourless needles, m. p. 183·5—184·5° (Found: C, 59·15; H, 3·9. $C_{10}H_8O_3N_2$ requires C, 58·8; H, 3·95%).

8-*Nitro*-4-*methoxyquinoline*.—Prepared from 4-chloro-8-nitroquinoline (0·2 g.), sodium methoxide (0·7 g.), and methanol (6 c.c.) (1 hour's reflux), 8-nitro-4-methoxyquinoline (0·12 g., 61%) crystallised from methanol in colourless prismatic needles, m. p. 180—181° (lit. m. p. 185—186°, 181—182) (Found: C, 58·3; H, 3·9; N, 13·6. Calc. for $C_{10}H_8O_3N_2$: C, 58·8; H, 3·95; N, 13·7%).

6-*Nitro*-4-*phenoxyquinoline*.—4-Chloro-6-nitroquinoline (7 g.) was heated at 120—140° (bath temp.) for 50 minutes with a solution of potassium hydroxide (1·95 g.) in phenol (70 g.). The mixture was cooled, diluted, basified with aqueous sodium hydroxide, and extracted with ether. Evaporation of the washed and dried extract gave 6-*nitro*-4-*phenoxyquinoline* (6·8 g., 76%) as cream-coloured needles, m. p. 117—118° from ligroin (b. p. 60—80°) (Found: C, 67·4; H, 4·0; N, 11·0. $C_{15}H_{10}O_3N_2$ requires C, 67·7; H, 3·8; N, 10·5%). At 90—95° the yield was 58%.

8-*Nitro*-4-*phenoxyquinoline*.—Interaction of the chloro-compound (10 g.), potassium hydroxide (2·7 g.), and phenol (100 g.) was complete in 1¾ hours at 90—95°; isolation as above gave 8-*nitro*-4-*phenoxyquinoline* (11 g., 86%) as colourless prismatic needles, m. p. 114—116° (sinters at 107°) from ligroin (b. p. 60—80°) (Found: C, 67·05; H, 3·9. $C_{15}H_{10}O_3N_2$ requires C, 67·7; H, 3·8%). Gouley *et al.* (*loc. cit.*) give m. p. 105—106° for an unanalysed specimen.

6-*Nitro*-2-*phenoxyquinoline*.—The product obtained by heating 2-chloro-6-nitroquinoline (1·5 g.), potassium hydroxide (0·41 g.), and phenol (15 g.) for 1 hour at 130—150° (lower temperatures gave a reduced yield) was isolated as above (1·3 g., 68%) and crystallised from ligroin (b. p. 60—80°) containing a little benzene; 6-*nitro*-2-*phenoxyquinoline* separated in amber-coloured needles, m. p. 151—152° (Found: C, 67·3; H, 3·4. $C_{15}H_{10}O_3N_2$ requires C, 67·7; H, 3·8%).

6-*Nitro*-4-*anilinoquinoline*.—A mixture of 4-chloro-6-nitroquinoline (0·2 g.), aniline (0·1 g.), acetone (1 c.c.), water (2 c.c.), and concentrated hydrochloric acid (1 drop) was refluxed for ½ hour. Removal of the acetone and basification with ammonia yielded 6-*nitro*-4-*anilinoquinoline* (0·25 g.; 98%), which formed orange prismatic needles, m. p. 183—184°, from alcohol (Found: N, 15·8. $C_{15}H_{11}O_2N_3$ requires N, 15·8%).

6-*Nitro*-4-*aminoquinoline*.—The phenoxy-compound (8 g.) and ammonium acetate (48 g.) were heated at 170—180° (bath temp.) for 25 minutes, cooled, diluted with water, and the solution basified. A solution of the crude product in 10% aqueous acetic acid (100 c.c.) was filtered and again basified, yielding 6-*nitro*-4-*aminoquinoline* (4·8 g., 84%), m. p. 311—312° (decomp.) after recrystallisation from alcohol, from which it formed deep orange irregular plates (Found: C, 57·1; H, 4·0; N, 22·4. $C_9H_7O_2N_3$ requires C, 57·1; H, 3·7; N, 22·2%). 6-*Nitro*-4-*acetamidoquinoline* (from the base and 5 parts of boiling acetic anhydride) crystallised from ethyl acetate in soft, almost colourless, prismatic needles, m. p. 228—229° (yield, 94%) (Found: N, 18·4. $C_{11}H_9O_3N_3$ requires N, 18·2%).

8-*Nitro*-4-*aminoquinoline*.—The temperature of a mixture of the phenoxy-compound (10 g.) and ammonium acetate (60 g.) was raised from 145° to 185° during 20 minutes, and kept at 185° for a further 10 minutes. Isolation as in the preceding example yielded 8-nitro-4-aminoquinoline (m. p. 215—222°, 6·65 g., 94%), which crystallised from ethyl acetate in irregular yellow leaflets, m. p. 229—230° [Gouley *et al.* (*loc. cit.*) give m. p. 232°] (Found: C, 57·4; H, 3·85; N, 22·2. Calc. for $C_9H_7O_2N_3$: C, 57·1; H, 3·7; N, 22·2%). 8-*Nitro*-4-*acetamidoquinoline* (yield, 80%), prepared from the base and boiling acetic anhydride, crystallised from slightly aqueous acetic acid in small colourless prisms, m. p. 290—292° (decomp.) (Found: N, 18·0. $C_{11}H_9O_3N_3$ requires N, 18·2%).

6-*Nitro*-2-*aminoquinoline*.—6-Nitro-2-phenoxyquinoline (0·3 g.) and ammonium acetate (3 g.) were kept at 210—225° for 4 hours (very little reaction was discernible after 1 hour). Extraction of the pro-

duct with 2N-hydrochloric acid yielded (acid-insoluble) unchanged phenoxy-compound (50 mg.), and (from the filtrate) 6-nitro-2-aminoquinoline (150 mg.) which formed orange prismatic needles, m. p. 256—257°, from alcohol (Found: C, 56·9; H, 4·1; N, 22·1. Calc. for $C_9H_7O_2N_3$: C, 57·1; H, 3·7; N, 22·2%). Tschitschibabin *et al.* (*loc. cit.*) give m. p. 251° and 261°. *6-Nitro-2-acetamidoquinoline* separated from benzene in colourless, hair-like needles, m. p. 231—233° (Found: N, 18·4. $C_{11}H_9O_3N_3$ requires N, 18·2%).

4 : 6-Bisacetamidoquinoline.—A solution of 6-nitro-4-aminoquinoline (2 g.) in glacial acetic acid (12 c.c.) was added at room temperature during 10 minutes to a stirred solution of stannous chloride dihydrate (8 g.) in hydrochloric acid (16 c.c.) and water (4 c.c.). The suspension was kept at 90° for 5 minutes and then basified (sodium hydroxide); 4 : 6-diaminoquinoline rapidly crystallised in colourless needles from the clear solution [1·4 g. (83%), m. p. 208° (decomp.) after being dried in an exhausted desiccator; the base turned red in air, and after 2 days had m. p. 195° (decomp.)]. The *dihydrochloride* separated from 2N-hydrochloric acid in colourless needles, m. p. 340—342° (decomp.) (Found: Cl, 29·7. $C_9H_9N_3,2HCl$ requires Cl 30·6%). When the crude base (1·4 g., m. p. 208°) was refluxed for ½ hour with acetic anhydride (14 c.c.), 4 : 6-*bisacetamidoquinoline* (2 g.) was formed (colourless irregular leaflets, m. p. 248·5—250°, from aqueous alcohol) (Found: C, 59·8; H, 5·8; N, 16·1. $C_{13}H_{13}O_2N_3,H_2O$ requires C, 59·7; H, 5·8; N, 16·1%). 4 : 6-Diacetamidoquinoline methiodide was prepared from the base (1·5 g.), methyl iodide (10 c.c.), and alcohol (40 c.c.) (1 hour's reflux); excess of methyl iodide was removed, the solid collected, and the filtrate again refluxed with more methyl iodide (10 c.c.) for 2 hours; total yield 78% (63% if only a single prolonged reflux was given). The salt formed yellow needles, m. p. 296—298° (decomp.), very easily soluble in water, and crystallising poorly from other solvents. The *methochloride* (from the iodide and silver chloride) crystallised well from alcohol or aqueous acetone in clusters of fine colourless needles, m. p. 318° (decomp.) (Found: C, 57·3; H, 5·5; N, 13·8. $C_{14}H_{16}O_2N_3Cl$ requires C, 57·2; H, 5·5; N, 14·3%).

6-Nitro-4-aminoquinoline Methiodide.—A suspension of 6-nitro-4-aminoquinoline (1·87 g., finely powdered) in alcohol (40 c.c.) and methyl iodide (13 c.c.) was refluxed gently for 7 hours, concentrated, and the solid collected [2·67 g. (80%), m. p. 303—304° (decomp.)]. Recrystallisation from water gave the *salt* as pale orange, lustrous, prismatic needles, m. p. 301—303° (decomp.) (Found: C, 36·6; H, 3·2; N, 12·9. $C_{10}H_{10}O_2N_3I$ requires C, 36·3; H, 3·0; N, 12·7%).

8-Nitro-4-aminoquinoline Methiodide.—A solution of 8-nitro-4-aminoquinoline (0·5 g.) in alcohol (50 c.c.) and methyl iodide (15 c.c.) was refluxed for 6 hours. Concentration and addition of ether gave *8-nitro-4-aminoquinoline methiodide* [0·4 g., m. p. 247—251° (decomp.)], which separated from water in red prismatic needles, m. p. 257—258° (decomp.), apparently as a hydrate, but satisfactory analytical data could not be obtained (Found: C, 34·2, 33·8; H, 2·7, 2·6; N, 12·95. $C_{10}H_{10}O_3N_3I,H_2O$ requires C, 34·4; H, 3·45; N, 12·0%); occasionally this substance crystallised in amber needles, m. p. 242° (decomp.), which were readily convertible into the higher-melting form by crystallisation from water.

6-Nitro-1-methyl-4-quinolone.—(*a*) Methyl sulphate (0·22 c.c.) was added to a solution of 6-nitro-4-hydroxyquinoline (0·2 g.) in aqueous potassium hydroxide (1·9%, 4·8 c.c.) at 50°. At the end of the reaction, excess of alkali was added, and the *6-nitro-1-methyl-4-quinolone* (0·21 g.) recrystallised from alcohol, from which it separated in yellow prismatic needles, m. p. 233—234° (Found: C, 58·9; H, 4·0. $C_{10}H_8O_3N_2$ requires C, 58·8; H, 3·95%).

(*b*) A solution of 6-nitro-4-aminoquinoline methiodide (0·3 g.) in water (8 c.c.) was refluxed for 1 hour with 10% aqueous sodium hydroxide (1 c.c.) (ammonia evolved). Recrystallisation of the product from alcohol gave pale orange prismatic needles (0·17 g.), m. p. 231—233° alone and when mixed with the sample described in (*a*).

1-Methyl-4-quinolone from 4-Aminoquinoline Methiodide.—The methiodide, prepared from the components in boiling ethanol, had m. p. 226—228° (Claus and Frobenius, *J. pr. Chem.*, 1897, **56**, 181, give m. p. 224°). Ammonia was evolved when it (2 g.) was refluxed in aqueous solution (10 c.c.) for ½ hour with sodium hydroxide (5 c.c. of 10%); extraction with chloroform and crystallisation of the product from benzene gave colourless prisms, m. p. 150—152°, of 1-methyl-4-quinolone (picrate, m. p. 227—229°) alone and when mixed with an authentic sample prepared from 4-hydroxyquinoline and methyl sulphate in sodium hydroxide solution.

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350. *The Growth of Titanium Nitride on Hot Filaments.*

By F. H. POLLARD and P. WOODWARD.

The conditions for the deposition of titanium nitride on hot filaments in a continuous-flow process have been studied, using the decomposition of halide in the presence of nitrogen and hydrogen. Optimum conditions for the deposition are: filament temperature $\simeq$ 1450°, total reaction pressure 30—40 cm. Hg, titanium chloride at 36° (vapour pressure $\simeq$ 17 mm.), and a gaseous mixture of equal quantities of nitrogen and hydrogen. The rate of flow is unimportant. The effect of the various factors on the nature of the deposit is discussed.

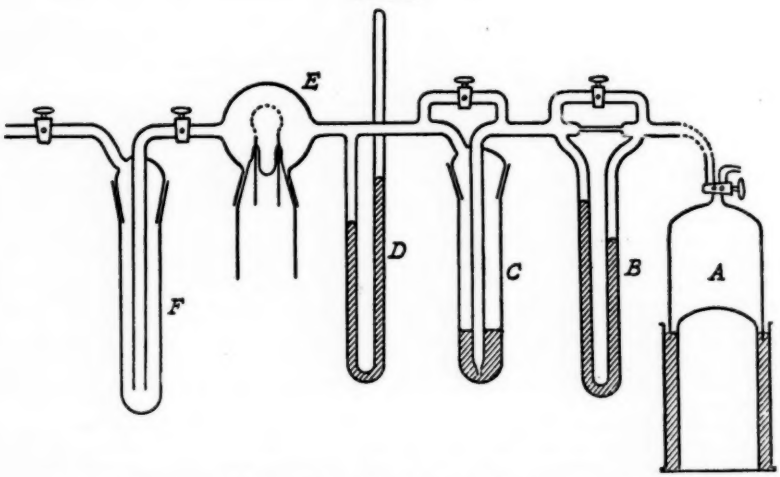
PREVIOUS work on the nitrides, carbides, and borides of the transition elements has been summarised by Becker (*Physikal. Z.*, 1933, **34**, 185; "Hochschmelzende Hartstoffe und ihre Technische Anwendung," Verlag Chemie, Berlin, 1935), but the most recent development has been given by Moers (*Z. anorg. Chem.*, 1931, **198**, 243), in which a crystalline product is grown

on a hot filament from the gaseous phase. For example, to form a nitride, a volatile halide of the metal can be reduced in presence of nitrogen. This technique has also been applied in the production of high-melting metals by Van Arkel (*Metallwirts.*, **1934**, **13**, 405), and the production of pure boron by Laubengayer, Hurd, Newkirk, and Hoard (*J. Amer. Chem. Soc.*, **1943**, **65**, 1924).

Experimental.

A continuous-flow method was found most suitable for the investigation of a reaction with so many variable factors, and the apparatus in its final form is illustrated in Fig. 1. The gasholder *A* contains any desired mixture of nitrogen and hydrogen at a pressure slightly greater than atmospheric. This can be pumped through the apparatus at a rate measured by the difference in pressure across the capillary in the manometer *B*, with butyl phthalate as the manometric liquid. The gases then bubble through the trap *C* containing titanium tetrachloride surrounded by a water-bath at a known temperature; it is assumed that the rate of flow is sufficiently slow for saturation to occur. The mixture then passes into the reaction vessel *E* containing the heated filament. The total pressure in *E* is measured by the mercury manometer *D*, the surface of the mercury being protected by a thin film of butyl phthalate. Reaction occurs on the hot filament, but owing to the extensive decomposition of the tetrachloride into lower chlorides on the vessel walls, it is impossible to measure the temperature of the filament with an optical pyrometer. It is therefore necessary to estimate this temperature by means of the current through the filament, and a calibration must be carried out previously under similar conditions, but in absence of titanium chloride vapour. It is unfortunate that extremely accurate control is not possible by this method, since the formation of the deposit is markedly dependent on the filament temperature.

Fig. 1.



In any experiment, the pressure and rate of flow are adjusted as necessary, the titanium chloride by-pass tap being kept open until steady conditions are reached. This tap is then closed, forcing the gases to bubble through the liquid, and the duration of the experiment is measured from this point.

The pressure indicated by the flowmeter *B* is not an absolute measure of the quantity of gas passing per unit time; this value depends also upon the total pressure in the reaction vessel. The Poiseuille formula shows that the quantity of gas flowing through a capillary under isothermal conditions is proportional to $p_2^2 - p_1^2$, where p_1 and p_2 are the pressures at the two ends of the capillary. Hence a constant value of $(p_2 - p_1)$ will give a constant rate of flow only if $(p_2 + p_1)$ is also constant. Thus, the rates of flow for two reactions are the same only if the total time is the same. This consideration applies when comparing reactions at different pressures.

The filaments are of tungsten or tantalum of various thicknesses. Fine tantalum wire can be spot-welded directly on to the thick tungsten leads, but tungsten filaments require a short nickel bridge. A.C. at low voltage is used for heating, currents up to 30 amps. being obtained from a Variac transformer. After adjusting the voltage to give the required initial current (and temperature), no further adjustments are made, but since nitrides of this type are good conductors, deposition of nitride causes a reduction of resistance in the filament and consequent increase in current. Thus, it is possible to estimate the rate of growth from the rate of increase of current under constant voltage. The total increase of current is not, however, an accurate measure of the quantity of nitride produced, since the actual increase will depend upon the crystalline structure of the deposit. Of two wires, coated with equal quantities of nitride, one having few large crystals and the other having many small ones, the former will have a smaller "effective" diameter than the latter—see Fig. 2, in which (*b*) would give a larger current increase than (*a*). All samples are therefore weighed to estimate the quantity of nitride produced.

Exit gases from the reaction are passed through a liquid-air trap *F* and a soda–lime tube to remove hydrogen chloride as completely as possible.

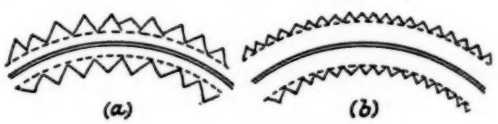
Experimental Results.—The variables investigated were: (1) The proportion of nitrogen to hydrogen in the gaseous mixture used. (2) The rate of flow of gases through the apparatus. (3) The temperature

of the titanium chloride bath, *i.e.*, the concentration of $TiCl_4$ vapour in the reaction mixture. (4) The total pressure in the reactions vessel. (5) The temperature of the filament. (6) The nature of the filament.

Initial Conditions.—A few preliminary experiments indicated that a deposit of nitride as well-formed, golden-yellow, cubic crystals can be obtained by using a total reaction pressure of 40 cm., a rate of flow of 4·5 cm. pressure of butyl phthalate, a titanium chloride bath at 18°, and a filament of tungsten, diameter 0·25 mm., length 2·5 cm., with an initial current of about 10 amps. These being used as the initial conditions, various modifications could be effected, and resulting differences in the product observed.

Effect of the N : H *Ratio.*—Experiments using mixtures of 3 : 1, 1 : 1 and 1 : 3 (N : H) revealed that, in general, equal volumes of nitrogen and hydrogen give the largest yield. Moreover, using this mixture, the yield is not affected by the position or orientation of the filament.

FIG. 2.



The Effect of Temperature of the Filament.—Experiments were carried out with the same initial conditions, but using a gaseous mixture of equal volumes of hydrogen and nitrogen. The filament temperature was first calibrated by means of an optical pyrometer, and a series of experiments was then carried out with different starting currents. The results are collected in the following table. There is a

	Initial current, amps.	Approx. initial temp.	Final current, amps.	Yield, mg.	Nature of deposit.
(1)	8	1050°	9	8	Dark purple and grey; microcrystalline
(2)	9	1250	18	30	Multicoloured small crystals; nitride crystal structure
(3)	10	1450	17½	27	Bronze yellow; fairly large crystals
(4)	11	1600	13½	4	Deposit at sides of bow only

narrow optimum range of temperature within which the nitride can be produced. If the temperature is lower than this value the crystals are black, and probably consist of pure metal. With an increase in temperature, the crystals become larger until an upper limit is reached, above which no deposit at all is obtained.

The multicoloured exterior which is sometimes obtained is probably due to a coating of lower chlorides of titanium deposited during the final pumping-out of the reaction vessel. The coloured layer can be removed with hot water, and breakage of a sample reveals a completely yellow cross-section except for the tungsten core.

A pointed filament (in place of the usual bow shape) gave a deposit only in the region away from the point. This is caused by the critical temperature being exceeded in the locality of the point. Apart from exceptional temperature effects of this kind, however, the shape does not markedly alter the nature of the deposit.

Effect of the Concentration of Titanium Chloride Vapour.—Results are summarised in the following table. It would appear that 36° is about the optimum temperature for the titanium chloride, equal

Temp. of $TiCl_4$ bath.	Initial current, amps.	Final current, amps.	Increase, amps.	Yield, mg.
0°	9½	<10	<½	Very small
10	9½	15	5½	18
18	9½	18½	9	29
29·5°	9½	18½	9	34
36	9½	22½	13	53
39	9½	18½	9	36

volumes of nitrogen and hydrogen being used. The size of the crystals deposited can be regulated by a judicious adjustment of the starting temperature. Higher relative concentrations of titanium chloride were not investigated because of the enormous amount of wall deposit produced.

Effect of the Rate of Flow and Total Reaction Pressure.—It was found that variation of the rate of flow by a factor of 3 produced no appreciable change, either in the amount of the deposit or in its characteristics. There is no doubt, however, that a pressure of 30—40 cm. is much more favourable for deposition than a pressure of about an atmosphere. The following table gives data to illustrate these facts.

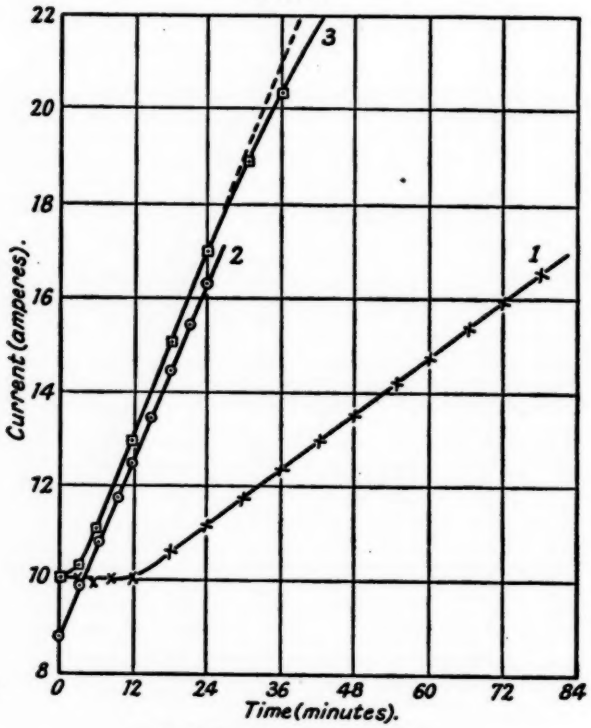
Analysis.—A sample of golden-yellow crystals analysed colorimetrically (by oxidation with hydrogen peroxide to the pertitanate) showed a titanium content agreeing with TiN within the limits of the method (about 2%). Titanium nitride is dissolved by fused potassium hydrogen sulphate.

Effect of the Nature of the Filament.—Two variables were investigated : (*a*) the chemical nature of the filament, (*b*) its physical condition. (*a*) Tungsten and tantalum filaments were used—tungsten in thicknesses of 0·2—0·35 mm., tantalum 0·2 mm. only. The qualitative result was the same in all cases, although the induction period before deposition begins tends to be longer for tantalum than for tungsten. In general, also, thin wires give a more even deposit than thick ones. (*b*) It was found that " aged "

Total press., cm. Hg.	Rate of flow.*	Current increase, amps.	Yield, mg.	Duration of expt., mins.
50	3·0	4·7	17	51
,,	4·5	4·4	18	35
,,	6·0	4·3	~16	25
,,	7·8	3·3	14	20
40	2·0	9·5	37	100
30	3·0	9·6	40	75
,,	6·2	11·3	44	40
,,	10·5	12·2	44	25
76	1·5	2·5	12	60
,,	3·0	2·7	13	40
,,	4·5	2·5	13	25
,,	6·0	2·5	14	15

* In cm. of butyl phthalate.

FIG. 3.



Variation of current with time.

	Press, cm.	$N_2 : H_2$.	Flow, cm.	$TiCl_4$.
1	40	1 : 1	2·3	21°
2	30	1 : 1	10·5	36
3	30	1 : 1	6·2	36

filaments (Langmuir, *Physical Rev.*, 1923, **22**, 374) tend to give a much more coherent and continuous layer of nitride than filaments which had not been so treated, but in no case could single-crystal coatings of nitride be produced. The currents necessary to produce any given temperature in a vacuum were calculated, Langmuir's tables being used with suitable corrections for end losses (Jones and Langmuir, *Gen. Elect. Rev.*, 1927, **30**, 311; Langmuir, Maclane, and Blodgett, *Physical Rev.*, 1930, **35**, 478). Electrical heating with A.C. can be considered equivalent to non-electrical heating (Johnson, *ibid.*, 1938, **54**, 459).

Discussion.—The rôle of the filament. Fig. 3 shows current–time curves during deposition under constant voltage. The induction period is probably controlled by the state of the filament, which may not only require cleaning but also etching (Taylor and Langmuir, *ibid.*, 1933, **44**, 423) to give a spacing more nearly equal to that of titanium nitride. Single-crystal filaments are said to give " einkrystalline " deposits (Fischvoigt and Koref, *Z. techn. Physik*, 1925, **6**, 296), but we have been unable to confirm this.

The linear increase indicates a constant rate of deposition of titanium and nitrogen, and the fall-off from linearity is explained by the critical temperature effect.

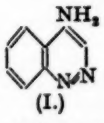
Mechanism. A likely mechanism is that the tetrachloride in presence of hydrogen is decomposed to the metallic state, and that nitride formation occurs simultaneously. Formation of complexes with ammonia, as described by Brager (*Acta Physicochim. U.R.S.S.*, 1939, **10**, 593; 1941, **14**, 297) at lower temperatures, seems improbable here. Preliminary experiments indicate that titanium nitride is unstable in a vacuum at 1500° and this is probably the cause of the critical temperature effect.

THE UNIVERSITY, BRISTOL. [*Received, December 22nd*, 1947.]

NOTES.

4-*Aminocinnoline*. By KINGSLEY BAKER.

No simple amino-derivatives of cinnoline have been described. 4-*Aminocinnoline* (I) has now been prepared by the action of alcoholic ammonia on 4-chlorocinnoline.


(I.)

4-Hydroxycinnoline (1 g.; Leonard and Boyd, *J. Org. Chem.*, 1946, **11**, 409) was refluxed with phosphorus oxychloride (5 ml.) for 30 minutes. The greater part of the unused reagent was removed by vacuum distillation, and the residue was poured on ice, made alkaline with sodium hydrogen carbonate, and extracted thrice with ether. The dried extract yielded 0·92 g. of crude 4-chlorocinnoline, m. p. *ca.* 60°. This was placed in a glass-lined steel tube with alcoholic ammonia (40 ml., saturated at − 8°) and copper acetate (trace) and heated in an oil-bath kept at 170° for 8 hours. The product was taken to dryness and extracted thrice with N-acetic acid. The acid extracts were treated with excess sodium hydroxide, giving 0·37 g. (39% yield, calculated on hydroxycinnoline) of pale yellow crystals, m. p. 208—210°, almost insoluble in cold water. Two recrystallisations from water produced 4-aminocinnoline as a light cream coloured microcrystalline powder, m. p. 209·5—210·5°, insoluble in ether, light petroleum, or benzene. It is sparingly soluble in boiling chlorobenzene with a gradient. The solutions show no fluorescence (Found: C, 65·7; H, 4·9; N, 28·7. $C_8H_7N_3$ requires C, 66·2; H, 4·9; N, 28·9%).

Unlike cinnoline itself, which is decomposed by air in a few minutes, this substance is quite stable and can be boiled with 2·5N-sodium hydroxide without loss of ammonia.

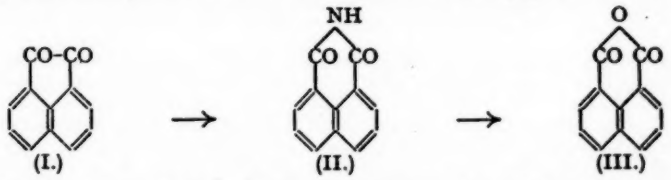
The author wishes to thank Dr. Adrien Albert for his interest and help, and Miss J. Fildes for the microanalysis.—UNIVERSITY OF SYDNEY. [*Received, October* 28th, 1947.]

Some Examples of the Schmidt Rearrangement. By W. G. H. EDWARDS and V. PETROW.

IN the course of other work we have had occasion to apply the Schmidt rearrangement (*Ber.*, 1924, **57**, 704; 1925, **58**, 2413) to a variety of compounds. The results, briefly outlined below, illustrate the highly specific character of this reaction.

Compounds containing a carbonyl grouping and a hetero-atom in the same ring in general failed to react. Experiments with oxindole, *N*-hydroxyoxindole, and 5-nitro-oxindole were not successful, the starting materials being recovered unchanged. *N*-Acetylindoxyl gave a dark amorphous solid which resisted all attempts at purification. Benz*iso*oxazolone and coumaranone were also recovered unchanged. The conversion of isatin into anthranilamide, reported by Caronna (*Gazzetta*, 1941, **71**, 585), has been confirmed and extended to the facile preparation of 4-chloro-, 6-chloro-, and 5-nitro-anthranilamides from the appropriate isatins. Isatin-7-carboxylic acid failed to react, a result which may be related to the depressed ketonic character of the β-carbonyl group owing to the proximity of the acid grouping.

Acenaphthenone gave a tarry product that could not be purified. Acenaphthenequinone (I) gave naphthalic anhydride (III), probably formed *via* (II) followed by hydrolysis by the sulphuric acid present in the reaction. An attempt to prepare the inaccessible 3-aminoacenaphthene from 3-acetylacenaphthene, readily obtained by the method of Fieser and Hershberg (*J. Amer. Chem. Soc.*, 1939, **61**, 1278), gave a mixture of 3-amino- and 3-acetamido-acenaphthenesulphonic acid. The relationship between the two compounds was demonstrated by acetylation of the former to a product whose S-*benzylisothiouronium* salt proved identical with that of the latter acid. 3-Acetamidoacenaphthene, it may be added, has been found to undergo rapid sulphonation in concentrated sulphuric acid at 0°. Attempts to carry out the rearrangement in syrupy phosphoric acid failed, unchanged ketone being recovered.



(I.) (II.) (III.)

Experimental.—Melting points are corrected. Semi-microanalyses are by Mr. S. Bance, B.Sc., A.R.I.C., Analytical Division, May and Baker Ltd.

The Schmidt rearrangements were carried out as follows. A solution of the compound in 10 vols. of concentrated sulphuric acid at 0° was treated with 1·1 equivs. of sodium azide, dissolved in the minimum quantity of distilled water and added dropwise with stirring over a period. After standing overnight, the mixture was poured on 10 times its weight of crushed ice, neutralised as necessary, and the product collected and purified.

5-Nitroanthranilamide, obtained by neutralisation of the product from the Schmidt rearrangement, formed orange needles from water (42%), m. p. 236° (Found: C, 46·5; H, 4·0; N, 23·5. Calc. for $C_7H_7O_3N_3$: C, 46·5; H, 3·9; N, 23·1%).

4-Chloroanthranilamide, prepared from 6-chloroisatin, formed prisms from ethanol (80%), m. p. 181° (Found: C, 49·5; H, 4·1; N, 16·3; Cl, 20·8. Calc. for $C_7H_7ON_2Cl$: C, 49·3; H, 4·1; N, 16·4; Cl, 20·8%), not depressed in admixture with an authentic specimen. The latter was obtained by the action of ammonia (5 ml.; *d* 0·88) on methyl 4-chloro-2-aminobenzoate (1 g.) in a sealed tube for 16 hours at 100—110°, m. p. 181° (Found: C, 49·5; H, 4·3; N, 16·5%). 4-Chloroisatin gave 6-chloroanthranilic acid (40%), colourless plates from benzene, m. p. 132°.

3-*Acetylacenaphthene experiments.* 3-Acetylacenaphthene (5 g.) in concentrated sulphuric acid (50 ml.) at 0° was treated with stirring with sodium azide (1·85 g.) in water (7·5 ml.). After 2 hours, ice (50 g.) was added, and the mixture filtered and washed with water (15 ml.); unchanged ketone (600 mg.) was then recovered. On mixing the filtrate and washings, a buff crystalline solid (2·1 g.) was deposited. It was collected, washed with acetone, and identified as 3-acetamidoacenaphthenesulphonic acid by conversion into the S-*benzylisothiouronium* salt, needles from 50% ethanol, m. p. 226° (decomp.) (Found: C, 55·8; H, 5·3; N, 8·9; S, 13·8. $C_{22}H_{22}O_4N_3S_2,H_2O$ requires C, 55·7; H, 5·3; N, 8·9; S, 13·5%). The acid filtrates (above) deposited a buff solid (900 mg.) on standing, which was evidently the 3-aminoacenaphthenesulphonic acid, as after acetylation it gave the same *S*-benzyl*iso*thiouronium salt, m. p. 226°.

The authors thank Dr. A. J. Ewins, D.Sc., F.R.S., and the Directors of Messrs. May and Baker Ltd. for research facilities generously placed at the disposal of one of them (W. G. H. E.).—May and Baker Ltd., Dagenham. Queen Mary College (University of London), E. 1. [*Received, October 30th,* 1947.]

The Preparation of 4 : 4′-*Diamino- and* 4 : 4′-*Dihydroxy*-2 : 2′-*dinaphthyl.* By Herbert H. Hodgson and Stanley Dixon.

The initial material for these preparations was the 4 : 4′-dinitro-2 : 2′-dinaphthyl prepared by Hodgson and Elliott (*J.*, 1937, 123) by the action of precipitated copper powder on 3-iodo-1-nitronaphthalene in nitrobenzene solution.

Reduction of 4 : 4′-*Dinitrodinaphthyl.*—A mixture of finely sieved 4 : 4′-dinitro-2 : 2′-dinaphthyl (10 g.), glacial acetic acid (100 c.c.), and hydrochloric acid (20 c.c., *d* 1·18) was reduced at the boil until colourless by the gradual addition of zinc dust (20 g.). During the reduction a heavy white precipitate of a zinc chloride double compound separated. After being cooled and diluted with water, the mixture was treated with aqueous sodium hydroxide in sufficient amount to dissolve all the zinc hydroxide. The precipitated 4 : 4′-*diamino*-2 : 2′-*dinaphthyl* was filtered off, and the filtrate extracted with chloroform to recover a small amount in solution; yield, 6·5 g. (78·7%). The diamine crystallised from a mixture of chloroform and light petroleum in white needles, m. p. 131° (Found: N, 9·7. $C_{20}H_{16}N_2$ requires N, 9·86%); it was only very sparingly soluble in water, but soluble in all the more common organic solvents except light petroleum, in which it was almost insoluble. The *dihydrochloride* was obtained in colourless needles by addition of hydrochloric acid (*d* 1·18) to a solution of the diamine in glacial acetic acid; it was almost completely insoluble in water; m. p. 308° (Found: HCl, 19·9. $C_{20}H_{16}N_2,2HCl$ requires HCl, 20·4%).

4 : 4′-*Diacetamido*-2 : 2′-*dinaphthyl* was formed when the diamine (0·5 g.) was heated with acetic anhydride (10 c.c.) on the water-bath for 15 minutes. On cooling, the diacetyl compound crystallised out and was all recovered (0·6 g., 92% yield) by dilution with water; it crystallised from glacial acetic acid in colourless needles, m. p. 340° (Found: N, 7·4. $C_{24}H_{20}O_2N_2$ requires N, 7·6%).

4 : 4′-*Dibenzamido*-2 : 2′-*dinaphthyl* was formed when the diamine was dissolved in pyridine (10 c.c.) and the solution heated with benzoyl chloride (0·8 g.) on the water-bath for 30 minutes, then diluted with water, and the separated benzoyl derivative filtered off, dried, and crystallised from nitrobenzene from which it separated (0·65 g., 75% yield) in colourless needles, m. p. 319° (Found: N, 5·5. $C_{34}H_{24}O_2N_2$ requires N, 5·7%).

4 : 4′-*Bistoluene*-p-*sulphonamido*-2 : 2′-*dinaphthyl* was formed when the solution of the diamine (0·5 g.) in pyridine (10 c.c.) was heated at the boil for 15 minutes with toluene-*p*-sulphonyl chloride (1·2 g.), then cooled, diluted with water, and the tarry precipitate hardened by washing it with dilute hydrochloric acid; the compound crystallised from nitrobenzene in colourless needles, m. p. 289° (Found: N, 4·5. $C_{34}H_{28}O_4N_2S_2$ requires N, 4·7%), in a yield of 0·8 g. (75·5%).

Preparation of 4 : 4′-*Dihydroxy*-2 : 2′-*dinaphthyl.*—A solution of 4 : 4′-diamino-2 : 2′-dinaphthyl (5 g.) in glacial acetic acid (15 c.c.) was stirred vigorously into 10% aqueous sulphuric acid (150 c.c.) to obtain a fine suspension of the insoluble sulphate. The mixture was cooled with ice and diazotised at 0° by the addition of a solution of sodium nitrite (2·45 g.) in water (50 c.c.). The diazo-solution was filtered, and the filtrate gradually raised to the boil; a vigorous evolution of nitrogen occurred, and 4 : 4′-*dihydroxy*-2 : 2′-*dinaphthyl* was precipitated. When the decomposition was complete, the precipitate was filtered off and extracted with hot 5% aqueous sodium hydroxide to remove the dihydroxy-compound, which was reprecipitated from the filtered extract by addition of acid, removed by

filtration, washed until acid-free, and recrystallised from 50% aqueous methanol. It formed colourless plates, m. p. 217° (Found: C, 83·8; H, 4·8. $C_{20}H_{14}O_2$ requires C, 83·9; H, 4·9%); yield, 3·8 g. (77%).

The authors thank Imperial Chemical Industries Ltd., Dyestuffs Division, for gifts of chemicals.—
TECHNICAL COLLEGE, HUDDERSFIELD. [*Received, November 3rd*, 1947.]

The Condensation of p-*Dimethylaminobenzaldehyde with* 4-*Methylpyrimidine Derivatives.* By D. M. BROWN and W. C. J. ROSS.

Ross (this vol., p. 1128) reported an unsuccessful attempt to prepare 4-styryl derivatives of uracil. When 4-methyluracil was heated with benzaldehyde or *p*-nitrobenzaldehyde in the presence of zinc chloride, condensation probably occurred at the 5-position in the pyrimidine nucleus with the formation of a xanthine-like compound; however, 5-nitro-4-methyluracil readily condensed with these aldehydes to give the required styryl derivatives. When dimethylaminobenzaldehyde was heated with 4-methyluracil in the presence of zinc chloride no definite product was obtained, but when they were heated together in aniline solution 4-(p-*dimethylaminostyryl*)*uracil* was formed. This method of condensation does not appear to have been used previously for the preparation of styrylpyrimidines. The reaction involves the elimination of aniline between the anil of the aldehyde and methyluracil, and when this anil was heated with 4-methyluracil at 180° the same condensation product was obtained, but it was more convenient to carry out the reaction as described below. Benzaldehyde did not condense with 4-methyluracil under these conditions. Chlorination of 4-(*p*-dimethylaminostyryl)uracil using phosphorus oxychloride afforded 2 : 6-*dichloro*-4-(p-*dimethylaminostyryl*)*pyrimidine*; this did not react with a solution of ammonia in methanol at the boiling point of the solvent, but at 150° a *chloro-amino*-derivative was formed. An attempt to prepare 6-chloro-2-amino-4-(*p*-dimethylaminostyryl)pyrimidine for comparison with this monoamine was unsuccessful, since neither 2-amino-6-hydroxy-4-methylpyrimidine nor the corresponding 6-chloro-derivative could be condensed with *p*-dimethylaminobenzaldehyde. 2-Thio-4-methyluracil condensed with *p*-dimethylaminobenzaldehyde in aniline solution to give 2-*thio*-4-(p-*dimethylaminostyryl*)*uracil*, and 6-hydroxy-4-methylpyrimidine similarly reacted to give 6-*hydroxy*-4-(p-*dimethylaminostyryl*)*pyrimidine*. Chlorination of the last compound yielded 6-*chloro*-4-(p-*dimethylaminostyryl*)*pyrimidine*, and this reacted with piperidine to give 6-*piperidino*-4-(p-*dimethylaminostyryl*)*pyrimidine*.

4-(p-*Dimethylaminostyryl*)*uracil*.—4-Methyluracil (25 g.), *p*-dimethylaminobenzaldehyde (50 g.), and aniline (400 c.c.) were heated under reflux for 24 hours. The water formed at the start of the reaction was removed by distillation. The cooled mixture was diluted with an equal volume of methanol, and the solid which separated washed with methanol and then ether (11·4 g. of amorphous material). The product was obtained in small yellow plates by dissolving it in hot acetic acid and then diluting with methanol; m. p. 325—330°, after a second crystallisation (Found: C, 65·0; H, 5·7; N, 16·6. $C_{14}H_{15}O_2N_3$ requires C, 65·4; H, 5·9; N, 16·3%). The compound exhibits a yellow-green fluorescence in ultra-violet light.

2 : 6-*Dichloro*-4-(p-*dimethylaminostyryl*)*pyrimidine*.—4-(*p*-Dimethylaminostyryl)uracil (1·5 g.) was heated under reflux with phosphorus oxychloride (9 c.c.) for 20 minutes. The mixture was poured on ice (100 g.) and extracted with ether, giving a yellow-green fluorescent solution from which orange crystals (0·75 g.) separated on evaporation. After recrystallisation from methanol, the *dichloro*-compound formed long flattened orange needles, m. p. 181—183° (Found: C, 57·0; H, 4·5. $C_{14}H_{13}N_3Cl_2$ requires C, 57·2; H, 4·5%).

Action of Ammonia on the Dichloro-compound.—The dichloro-compound (1·5 g.) was heated for 4 hours in a sealed tube at 140—150° (glycerol-bath temperature) with a solution of ammonia in methanol (30 c.c., saturated at room temperature). The orange solid gradually passed into solution, and later pale yellow crystals of the *monoamino*-compound separated from the hot solution. The product, after being washed with methanol, decomposed on heating and finally melted above 270° (Found: C, 61·4; H, 5·5. $C_{14}H_{15}N_4Cl$ requires C, 61·2; H, 5·5%).

2-*Thio*-4-(p-*dimethylaminostyryl*)*uracil*.—2-Thio-4-methyluracil (1·0 g.), *p*-dimethylaminobenzaldehyde (1·0 g.), and aniline (15 c.c.) were heated under reflux for 4 hours, and then the cooled mixture was diluted with an equal volume of methanol. The orange-red solid which separated was dissolved in dilute hydrochloric acid. The colourless solution was warmed and then saturated with sodium acetate. The *thio*-derivative which separated was washed well with warm water and finally with methanol. After being dried at 140°/0·001 mm. the compound decomposed above 280° without melting (Found: C, 61·6; H, 5·8. $C_{14}H_{15}ON_3S$ requires C, 61·5; H, 5·5%).

6-*Hydroxy*-4-(p-*dimethylaminostyryl*)*pyrimidine*.—6-Hydroxy-4-methylpyrimidine was liberated from the hydriodide (Gabriel and Colman, *Ber.*, 1899, **32**, 2930) by treatment of the aqueous solution with lead acetate. The free base (3·3 g.), *p*-dimethylaminobenzaldehyde (4·5 g.), and aniline (6 c.c.) were heated under reflux for 4 hours. The dark solution was cooled and diluted with methanol (6 c.c.). The *product* (0·9 g.) separated from acetic acid–methanol as a yellow crystalline powder, m. p. 295—297° (Found: C, 69·4; H, 6·2. $C_{14}H_{15}ON_3$ requires C, 69·7; H, 6·3%).

6-*Chloro*-4-(p-*dimethylaminostyryl*)*pyrimidine*.—The above hydroxy-compound (1·2 g.) and phosphorus oxychloride (6 c.c.) were heated under reflux for 1½ hours. The mixture was poured on ice and the solution was then basified with ammonia and extracted with benzene. After drying, the benzene solution was percolated down a column of activated alumina. Elution of the yellow band with more benzene followed by the evaporation of the solvent gave the *chloro*-compound which separated from benzene–light petroleum (b. p. 60—80°) in the form of yellow leaflets (0·3 g.), m. p. 192° (Found: C, 64·7; H, 5·5. $C_{14}H_{14}N_3Cl$ requires C, 64·7; H, 5·4%).

6-*Piperidino*-4-(p-*dimethylaminostyryl*)*pyrimidine*.—The chloro-compound (100 mg.) and piperidine (0·5 c.c.) were heated at the boiling point for 5 minutes. After the addition of water an oil separated. This solidified on standing, and was then washed, dried, and recrystallised from light petroleum (b. p.

60—80°), giving small pale yellow needles (75 mg. after two crystallisations), m. p. 126° (Found: C, 74·5; H, 7·6. $C_{19}H_{24}N_4$ requires C, 74·0; H, 7·8%).

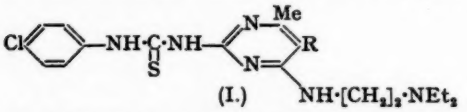
This investigation has been supported through grants made to the Royal Cancer Hospital (Free) by the British Empire Cancer Campaign, the Anna Fuller Fund, and the Jane Coffin Childs Memorial Fund. One of us (W. C. J. R.) wishes to thank the Sir Halley Stewart Trust for the award of a research Fellowship.—THE CHESTER BEATTY RESEARCH INSTITUTE, THE ROYAL CANCER HOSPITAL (FREE), FULHAM ROAD, LONDON, S.W. 3. [*Received, November 20th*, 1947.]

Symmetrically Disubstituted Formamidines. Formation by Raney-nickel Reduction of Thioureas.

By R. DEBRATH ASHWORTH.

THE use of Raney nickel alone (*i.e.*, in the absence of added hydrogen) as a reducing agent has been described by Mozingo (*J. Amer. Chem. Soc.*, 1943, **65**, 1013), who converted benzoylmethionine into α-benzamidobutyric acid, and more recently Wolfram and Karabino (*ibid.*, 1944, **66**, 909) have used this reagent for the reduction of thioacetals to the corresponding methylenic compounds. After the completion of the work now described a paper by Cattelain and Chabrier (*Bull. Soc. chim.*, 1940, **6**, 781) became available in which the reduction of certain monosubstituted thioureas by Raney nickel was described; *e.g.*, benzylthiourea yielded ammonia, methylamine, and toluene, whereas sodium α-mercaptocinnamate afforded β-phenylpropionic acid. There appeared to be no literature reference, however, to the reduction of *s*-disubstituted thioureas to formamidines.

The work described here was carried out on compounds of type (I) which were available as a result of an earlier antimalarial research (Ashworth, Crowther, Curd, and Rose, *J.*, this vol., p. 581). By reduction



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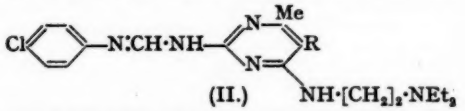


(II.)

of 4-2′-diethylaminoethylamino-2-*p*-chlorophenylthioureido-6-methylpyrimidine (I; R = H) with Raney nickel in cold ethyl acetate there was obtained a high yield of a compound $C_{18}H_{23}N_6Cl$, to which the structure (II; R = H) was assigned. To check the course of the reduction, thiocarbanilide was used as a model substance and under identical conditions gave a good yield of diphenylformamidine. The ultra-violet absorption spectrum of the new compound (II; R = H) shows a maximum at 2920 A., and the urea corresponding to (I; R = H) (Ashworth *et al.*, *loc. cit.*) shows a maximum at 2510 A., and this shift in the maximum is considered to be due to the introduction of a double bond into the molecule, most probably in conjugation with the phenyl nucleus.

Reduction of *p*-chlorophenylthiourea gave an unidentified basic liquid and no uniform product could be isolated from the attempted reduction of *N*-amidino-*N*′-*p*-chlorophenylthiourea. However, the Raney nickel reduction of 4-2′-diethylaminoethylamino-2-*p*-chlorophenylthioureido-6-methyl-5-ethylpyrimidine (I; R = Et) and of 2-*p*-chlorophenylthioureido-4 : 6-dimethoxypyrimidine gave the corresponding formamidines (II; R = Et) and *N*′-*p*-chlorophenyl-*N*-(4 : 6-dimethoxy-2-pyrimidyl)-formamidine, respectively.

Experimental.—*N*′-p-*Chlorophenyl*-N-(4-2′-*diethylaminoethylamino*-6-*methyl*-2-*pyrimidyl*)*formamidine* (II; R = H). 4-2′-Diethylaminoethylamino-2-*p*-chlorophenylthioureido-6-methylpyrimidine (9 g.) in ethyl acetate (40 c.c.) was shaken for 15 hours with Raney nickel (37 g. of alcoholic paste) at room temperature. The mixture was then raised to the boil, filtered, and allowed to cool. The crystalline *product* was collected, and the filtrate used to re-extract the nickel, a further small quantity being thus obtained (yield, 5·2 g.). After recrystallisation from ethyl acetate it had m. p. 174—175° (Found: C, 60·0, 59·8; H, 7·0, 7·0; N, 23·3. $C_{18}H_{23}N_6Cl$ requires C, 60·0; H, 6·95; N, 23·3%). The yield was not improved by the continuous passage of hydrogen through the solution, and the use of alcohol as solvent gave an impure product. No reduction occurred with palladium and hydrogen, and reduction with zinc dust and ammonium chloride gave a non-characterisable product.

NN′-*Diphenylformamidine.* *NN*′-Diphenylthiourea (5 g.) in ethyl acetate (20 c.c.) was treated with Raney nickel (20 g.) as in the preceding experiment. The product obtained by evaporating the ethyl acetate to dryness under dimished pressure was crystallised from light petroleum (b. p. 60—80°)–alcohol and then had m. p. 137—138° undepressed in admixture with authentic *NN*′-diphenylformamidine (Found: C, 79·2; H, 5·8; N, 14·5. Calc. for $C_{13}H_{12}N_2$: C, 79·6; H, 6·1; N, 14·3%).

N′-p-*Chlorophenyl*-N-(4-2′-*diethylaminoethylamino*-6-*methyl*-5-*ethyl*-2-*pyrimidyl*)*formamidine* (II; R = Et). 4-2′-Diethylaminoethylamino-2-*p*-chlorophenylthioureido-6-methyl-5-ethylpyrimidine (1 g.) was dissolved in ethyl acetate (8 c.c.) and shaken with Raney nickel (7·5 g. of alcoholic paste) and worked up as described above to give the *product* (yield, 0·2 g.), which had m. p. 149—151° after recrystallisation from light petroleum (b. p. 80—100°) (Found: C, 61·4; H, 7·2; N, 21·4. $C_{20}H_{27}N_6Cl$ requires C, 61·7; H, 7·3; N, 21·6%).

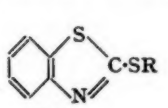
N′-p-*Chlorophenyl*-N-(4 : 6-*dimethoxy*-2-*pyridyl*)*formamidine.* 2-*p*-Chlorophenylthioureido-4 : 6-dimethoxypyrimidine (to be described in a forthcoming communication) (8·5 g.) was reduced in the usual way in ethyl acetate (70 c.c.) with Raney nickel (52·5 g. of alcoholic paste). The *product* (3·5 g.) was purified by crystallisation from alcohol and then had m. p. 178—179·5° (Found: C, 53·6; H, 4·5. $C_{13}H_{13}O_2N_4Cl$ required C, 53·3; H, 4·45%).

The author wishes to thank Dr. F. S. Cropper for the spectroscopic data and Dr. F. H. S. Curd for advice.—IMPERIAL CHEMICAL INDUSTRIES LTD., RESEARCH LABORATORIES, BLACKLEY, MANCHESTER, 9. [*Received, December 4th*, 1947.]

The Hydroxymethyl Derivative of 2-Mercaptobenzthiazole. By W. A. SEXTON and A. SPINKS.

2-MERCAPTOBENZTHIAZOLE and aqueous or alcoholic formaldehyde afford a hydroxymethyl derivative to which the structure 2-hydroxymethylthiobenzthiazole has been assigned (B.P. 361,971; Levi, *Gazzetta*, 1932, **62**, 775). The *S*-substituted structure has also been assumed for various derivatives such as the condensation products of 2-mercaptobenzthiazole with formaldehyde and amines (B.PP. 377,253, 410,454) and with formaldehyde and β-naphthol (B.P. 476,838), the reaction products of the hydroxymethyl compound with acyl halides and phenyl *iso*cyanate (B.P. 475,220), the condensation product of 2-mercaptobenzthiazole with "dimethylolurea" (bishydroxymethylurea) (B.P. 470,791) and the chloromethyl compound obtained with phosphorus trichloride (B.P. 475,221).

Although methylation of the thiol compound (I; R = H) with methyl sulphate and methyl iodide affords only a small proportion of the *N*-methyl derivative (II; R = Me) (Reed, Robertson, and Sexton,



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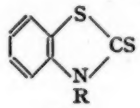


(II.)

J., 1939, 473), the facts that (i) 2-mercaptobenzthiazole shows in solution the structure (II; R = H) (Morton and Stubbs, *J.*, 1939, 1321), and (ii) the hydroxymethyl derivative is formed in the absence of alkali, caused us to question the hitherto accepted structure. We have therefore investigated the ultraviolet absorption spectra of the hydroxymethyl and the piperidinomethyl derivative of 2-mercaptobenzthiazole, and a comparison with the data provided by Morton and Stubbs (*loc. cit.*) shows clearly that they possess *N*-substituted structures (II; R = CH_2·OH and R = CH_2·NC_5H_{10}, respectively). The structures of the various derivatives described in the patents mentioned above therefore require appropriate modification.

2-Thio-3-hydroxymethylbenzthiazolone (II; R = CH_2·OH).—2-Mercaptobenzthiazole (34 g.) was boiled for 1¼ hours with alcohol (30 c.c.) and 37% formaldehyde (20 g.). On cooling, the crystalline product (33·7 g.) was separated, washed with 50% alcohol, and recrystallised from toluene (charcoal); m. p. 129—130° (Levi, *loc. cit.*, gives m. p. 125—130° for his "2-hydroxymethylthiobenzthiazole") (Found: N, 7·1; S, 32·15. $C_8H_7ONS_2$ requires N, 7·1; S, 32·5%). The *compound* resembles 2-thio-3-methylbenzthiazolone in its insolubility in concentrated hydrochloric acid. It is decomposed by boiling water and by cold dilute sodium hydroxide with regeneration of 2-mercaptobenzthiazole.

2-Thio-3-piperidinomethylbenzthiazolone (II; R = CH_2·NC_5H_{10}).—Piperidine (8·5 g.) was mixed with 37% formaldehyde (8·3 g.) and to the cooled mixture was added 2-mercaptobenzthiazole (16·7 g.) dissolved in acetone (80 c.c.). The yellow crystalline *compound* was separated and recrystallised; yellow prisms from ethyl acetate, m. p. 154—156°; B.P. 377,253 gives m. p. 159—161° for the alternative structure (Found: N, 10·2; S, 24·2. $C_{13}H_{16}N_2S_2$ requires N, 10·3; S, 24·3%).

2-Thio-3-chloromethylbenzthiazolone (II; R = CH_2Cl).—The foregoing hydroxymethyl compound (19·7 g.) was mixed with phosphorus trichloride (8·5 c.c.). After the initial vigorous reaction had subsided, ethylene chloride (25 c.c.) was added, and the mixture boiled for 20 minutes. The hot solution was decanted from gummy material, and the crystalline product which separated on cooling was recrystallised from benzene; pale yellow needles, m. p. 127—128°; B.P. 475,221 gives m. p. 127—128° for the supposed isomer (Found: S, 29·7; Cl, 16·2. $C_8H_6NClS_2$ requires S, 29·7; Cl, 16·5%). This *compound* contains a reactive halogen atom and tends to deteriorate in moist air.

Absorption Spectra.—These were determined in methyl-alcoholic solution, a Beckmann spectrophotometer being used. The values of the principal absorption bands are given below in comparison with those found by Morton and Stubbs (*loc. cit.*) for 2-mercaptobenzthiazole and its isomeric methyl derivatives. The general shape of the curves for the two new compounds and the position of the maxima showed such close agreement with those for (II; R = H or Me) that there was no doubt as to the essential identity of the absorbing system. A minor point was the failure to observe with our compounds the resolution of the band at λ = 236 mμ as recorded by Morton and Stubbs for (II; R = Me).

Compound.	$\lambda_{max.}$	log $\epsilon_{max.}$	Compound.	$\lambda_{max.}$	log $\epsilon_{max.}$
(II; R = H)	325	4·43	(II; R = Me)	324·5	4·41
	235	4·10		241	4·14
(II; R = CH_2·OH)	321	4·34		231	4·13
	236	4·41	(I; R = Me)	300·5	3·92
(II; R = CH_2·NC_5H_{10})	323	4·39		280	4·09
	236·5	4·14		224	4·36

The absorption of the chloromethyl compound in the same solvent was not determined owing to the reactivity of the halogen atom.—RESEARCH LABORATORIES, IMPERIAL CHEMICAL INDUSTRIES LTD., BLACKLEY, MANCHESTER. [*Received, December 9th*, 1947.]

Derivatives of 2 : 4-Dinitrophenylacetone. By J. S. MORLEY, J. C. E. SIMPSON, and O. STEPHENSON.

2 : 4-DINITROPHENYLACETONE, originally prepared from ethyl 2 : 4-dinitrophenylacetoacetate (Borsche, *Ber.*, 1909, **42**, 601), was also readily obtained from 2 : 4-dinitrophenylacetyl chloride and ethyl ethoxymagnesiomalonate (cf. Walker and Hauser, *J. Amer. Chem. Soc.*, 1946, **68**, 1386). Reduction yielded a nitro-amine which is probably *4-nitro-2-aminophenylacetone*, as diazotisation yielded a sparingly soluble

product which was not a diazonium salt but was possibly the related indazole. 2 : 4-Dinitrophenylacetoxime on reduction gave a product which diazotised normally but showed no tendency to cyclise to a dihydrocinnoline, and is therefore probably the *oxime* of 2-nitro-4-aminophenylacetone.

Condensation of 2-chloro-3 : 5-dinitrobenzoic acid with ethyl acetoacetate by the method of Borsche (*loc. cit.*) yielded *ethyl* 2 : 4-*dinitro*-6-*carboxyphenylacetoacetate* (I), from which the arylacetone could not be prepared by fission with concentrated sulphuric acid (Borsche, *loc. cit.*) or by other means. Treatment of (I) with acetic anhydride and with phenylsemicarbazide gave *products*, m. p. 124° and 256° respectively, and interaction of (I) and hydroxylamine yielded two *products*, m. p. 230° and 239°. The structures of these four products are unknown.

Contrary to the claim of Brown and Campbell (*J.*, 1937, 1699), 2 : 4-dinitrobenzoic acid could not be prepared by simultaneous nitration and oxidation of phenylacetic acid; several repetitions of their work gave 2 : 4-dinitrophenylacetic acid in good yield as the sole isolable product.

2 : 4-*Dinitrophenylacetic Acid*.—The reaction of phenylacetic acid with nitric acid (*d* 1·5) was carried out exactly as described by Brown and Campbell (*loc. cit.*), and also with longer periods of reflux (3 hours) and with more concentrated nitric acid (*d* 1·53). In each case the acid had m. p. 181—182° (efferv.) [Borsche (*Ber.*, 1909, **42**, 1310) gives m. p. 179° (decomp.)], unchanged by crystallisation from aqueous alcohol (150—154° when mixed with 2 : 4-dinitrobenzoic acid, m. p. 181—182°, prepared from 2 : 4-dinitrophenyl cyanide).

2 : 4-*Dinitrophenylacetone*.—The acid prepared as above (21 g.), phosphorus pentachloride (23 g.), and benzene (40 c.c.) were heated under reflux for ½ hour, and phosphorus oxychloride removed by repeated evaporation with benzene under reduced pressure. A solution of the residue in ether (200 c.c.) was added dropwise to a stirred solution prepared from magnesium (2·68 g.), ethyl malonate (17·6 g.), alcohol (12·5 c.c.), ether (50 c.c.), and carbon tetrachloride (0·25 c.c.). After ½ hour (reflux) the mixture was cooled and acidified (sulphuric acid), and the product was collected with ether, freed from solvent, and refluxed for 5 hours with a mixture of glacial acetic acid (30 c.c.), concentrated sulphuric acid (3·8 c.c.), and water (20 c.c.). Partial neutralisation of the ice-cold solution gave almost pure 2 : 4-dinitrophenylacetone (12·3 g.), m. p. 77—78° alone and mixed with authentic material (Borsche, *Ber.*, 1909, **42**, 601) after crystallisation from ether–ligroin (Found: C, 48·1; H, 3·75; N, 12·4. Calc. for $C_9H_8O_5N_2$: C, 48·2; H, 3·6; N, 12·5%).

4-*Nitro*-2-*aminophenylacetone*.—A suspension of the dinitro-ketone (0·84 g.) in alcohol (5 c.c.) and concentrated hydrochloric acid (1 c.c.) was treated during ¼ hour at 20—25° with a solution of stannous chloride (2·8 g.) in concentrated hydrochloric acid (5 c.c.). After 5 minutes at 30—35°, the clear red solution was diluted with water (5 c.c.), and the precipitated *amine* (0·44 g., m. p. 159—160°) recrystallised from water containing a drop of hydrochloric acid, from which it separated in long, silky orange needles, m. p. 161—162° (decomp.) (Found: C, 56·2; H, 4·7; N, 14·7. $C_9H_{10}O_3N_2$ requires C, 55·7; H, 5·2; N, 14·4%). The amine was prepared from the dinitro-ketone obtained from 2 : 4-dinitrophenylacetic acid and also from ethyl 2 : 4-dinitrophenylacetoacetate; it gave a red colour in very weakly alkaline, but was stable in faintly acid, solutions.

Oxime of 2-*Nitro*-4-*aminophenylacetone*.—A finely-divided suspension of 2 : 4-dinitrophenylacetoxime (2 g.) (Borsche, *Annalen*, 1912, **390**, 25) in alcohol (100 c.c.) and water (150 c.c.) was shaken for 1 hour with aqueous sodium hydrogen sulphide (from 0·7 g. of sodium hydroxide in 40 c.c. of water); the mixture was then heated on the water-bath until a clear red solution was obtained, and this was left at room temperature for 2 days. The *nitro-amine* which had then separated (0·57 g.) was crystallised from methanol, giving yellow needles, m. p. 205°, easily soluble in dilute mineral acids and in excess of sodium hydroxide (Found: C, 52·0; H, 5·1. $C_9H_{11}O_3N_3$ requires C, 51·65; H, 5·3%).

Ethyl 2 : 4-*Dinitro*-6-*carboxyphenylacetoacetate*.—A solution of ethyl acetoacetate (52 g.) in ether (350 c.c.) was treated with sodium (9·2 g.) followed by methyl alcohol (50 c.c.). A solution of 2-chloro-3 : 5-dinitrobenzoic acid (25 g.) in methyl alcohol (50 c.c.) and ether (250 c.c.) was added, and the whole heated under reflux for 3—4 hours. The solution was then washed with water and very dilute sodium hydroxide solution, the combined washings made acid to Congo-red, and the product crystallised (aqueous methanol, benzene–ligroin, and finally benzene); the *ester* (14—15 g.) separated in small colourless needles, m. p. 142—143°, giving a deep red colour with alcoholic ferric chloride (Found: C, 46·4; H, 3·6; N, 8·6. $C_{13}H_{12}O_9N_2$ requires C, 45·9; H, 3·55; N, 8·2%), easily soluble in aqueous sodium acetate and in methyl alcohol, moderately in benzene, and very sparingly in ligroin.

Reactions of Ethyl 2 : 4-*Dinitro*-6-*carboxyphenylacetoacetate*.—(*a*) The ester (0·75 g.) and acetic anhydride (1 g.) were refluxed for 10 minutes, solvent removed (reduced pressure), and the residue crystallised from ether and finally from ether–ligroin, from which the *product* separated in colourless crystals, m. p. 124° with previous softening, insoluble in dilute sodium carbonate (Found: C, 47·3; H, 3·1; N, 9·0. $C_{13}H_{10}O_8N_2$ requires C, 48·4; H, 3·1; N, 8·7%).

(*b*) The ester (0·2 g.) and phenylsemicarbazide (0·1 g.) were refluxed for ¾ hour in alcohol (6 c.c.) containing acetic acid (4 drops). After slight concentration, the solution was diluted with water (2 vols.), and the precipitated solid recrystallised from alcohol and finally from aqueous acetic acid, from which the *product* separated in fine yellow needles, m. p. 255—256° (decomp.) (Found: C, 52·7; H, 3·8; N, 15·5. $C_{20}H_{17}O_8N_5$ requires C, 52·7; H, 3·75; N, 15·4%).

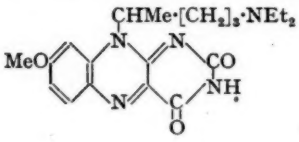
(*c*) A solution of the ester (3 g.), hydroxylamine hydrochloride (3 g.), and sodium acetate crystals (6 g.) in alcohol (35 c.c.) and water (100 c.c.) was heated under reflux for 4 hours, cooled, diluted with water, and made just acid to Congo-red with hydrochloric acid. The solid which separated was recrystallised from benzene–alcohol, yielding a *substance* which formed bright yellow needles, m. p. 239° (Found: C, 45·25; H, 2·8; N, 15·7. $C_{10}H_7O_6N_3$ requires C, 45·3; H, 2·65; N, 15·85%). The aqueous filtrate from this substance was extracted with ether; evaporation of the washed and dried extract yielded a second *substance*, sparingly soluble in benzene, which, after several crystallisations from benzene–alcohol, formed pale yellow needles, m. p. 230° (decomp.) (220° when mixed with the foregoing compound); it gave brownish-yellow solutions in alcohol and acetone, almost colourless in benzene, and deep red in aqueous or alcoholic sodium hydroxide (Found: C, 42·6; H, 2·3. $C_{11}H_7O_8N_3$ requires C, 42·7; H, 2·3%).

We are indebted to the Medical Research Council for a Research Studentship (J. S. M.).—DURHAM COLLEGES IN THE UNIVERSITY OF DURHAM. WARRINGTON YORKE DEPARTMENT OF CHEMOTHERAPY, LIVERPOOL SCHOOL OF TROPICAL MEDICINE. [*Received, December 12th*, 1947.]

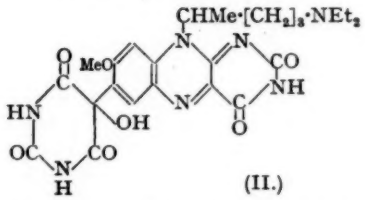
Contribution to the Chemistry of Synthetic Antimalarials. Part VII. The Reaction between Alloxan and 4-Amino-3-(4-diethylamino-1-methylbutylamino)anisole. By A. MCCOUBREY and W. WEBSTER.

VARIOUS 9-basically substituted *iso*alloxazines have been described recently (Hall and Turner, *J.*, 1945, 699; King and Acheson, *J.*, 1946, 681; Neeman, *ibid.*, p. 811; Adams, Weisel, and Mosher, *J. Amer. Chem. Soc.*, 1946, **68**, 883), and in view of this the following observation appears to be worthy of note.

During attempts to synthesise 7-methoxy-9-(4-diethylamino-1-methylbutyl)*iso*alloxazine (I) a product was isolated which appears to be a compound between alloxan and (I).



(I.)



(II.)

Condensation of alloxan and 4-amino-3-(4-diethylamino-1-methylbutylamino)anisole in cold acetic–boric acid solution in the dark resulted in the rapid development of a yellow colour with a pronounced green fluorescence. A yellow microcrystalline compound was isolated, the analysis of which indicated that it was a product formed from (I) and alloxan. While this work was in progress Kipnis, Weiner, and Spoerri (*J. Amer. Chem. Soc.*, 1947, **69**, 799) described the isolation of (I) which was obtained when condensation was effected in boiling acetic–boric acid solution. This work has now been repeated and confirmed, and the products have been compared and shown to differ in properties. The complex was stable to cold aqueous alkali and could not be extracted from alkaline solution by chloroform.

The condensation of alloxan with aromatic amines (Pellizarri, *Gazzetta*, 1887, **17**, 412) and with phenols (D.R.-P. 107,720, 115,817; Schwyzer, *Chem. Zentr.*, 1930, **54**, 839) to form aldol-type products furnishes a clue to the possible structure of the complex, and it is tentatively suggested that the product has the structure (II). It is noteworthy that the majority of the 9-basically substituted *iso*alloxazines described in the literature have been substituted in the 6-position—a possible reason why products similar to that now described have not been encountered in the *iso*alloxazine series.

Condensation of alloxan and 4-amino-3-(4-diethylamino-1-methylbutylamino)anisole in aqueous hydrochloric acid gave 7-methoxyalloxazine by cleavage of the side chain (cf. Hall and Turner, *loc. cit.*), and a similar substance was produced by condensation of 3 : 4-diaminoanisole with alloxan, though the possibility of formation of 6-methoxyalloxazine in this case is not precluded.

4-Nitro-3-(4-diethylamino-1-methylbutylamino)anisole.—3 : 4-Dinitroanisole (Kipnis, Weiner, and Spoerri, *J. Amer. Chem. Soc.*, 1944, **66**, 1446) (16 g.) and 4-diethylamino-1-methylbutylamine (15·8 g.) were heated in cymene (350 c.c.) under reflux until a vigorous reaction began. Heating was discontinued until the reaction moderated, and was then resumed until no more nitrous fumes were evolved. The mixture was cooled, and extracted with hydrochloric acid (15%; 3 portions of 100 c.c.), and the acid extract washed once with ether, then basified. The precipitated oil was taken up in chloroform, dried (K_2CO_3), and filtered. Removal of the solvent left a dark red oil (19 g.) which distilled at 175—180°/0·4 mm. The *picrate* crystallised from alcohol in yellow prisms, m. p. 111—112° (Found: N, 15·5. $C_{16}H_{27}O_3N_3,C_6H_3O_7N_3$ requires N, 15·6%).

Condensation of Alloxan and 4-Amino-3-(4-diethylamino-1-methylbutylamino)anisole.—4-Nitro-3-(4-diethylamino-1-methylbutylamino)anisole (19·3 g.) was reduced catalytically in alcohol (100 c.c.) at 20° and 5 atmospheres pressure using platinum oxide (5%); 95% of the theoretical amount of hydrogen was absorbed during 1·75 hours. The solvent was removed from the filtered solution; the residue distilled at 154—165°/0·03 mm. Yield, 16·3 g.

The diamine (8·7 g.) dissolved in glacial acetic acid (130 c.c.) was added to a solution of alloxan (8·7 g.) and boric acid (17 g.) in the same solvent (900 c.c.), and the mixture was left at room temperature overnight. The yellow solution, which had a pronounced green fluorescence, was evaporated under reduced pressure, and the sticky residue washed repeatedly with ethyl acetate and then dried over potassium hydroxide in a vacuum. The residue (17 g.) separated from methanol–ethyl acetate as an orange microcrystalline powder (12·5 g.) which by repeated crystallisation became insoluble in methanol and was finally purified by solution in a minimal amount of water and addition of warm methanol. Orange micro-crystals slowly separated which decomposed without melting at 240°, and were readily soluble in water, giving a yellow solution with a strong green fluorescence [Found: C, 49·6; H, 6·0; N, 16·9; OMe, 5·7; H_2O (Karl Fischer), 11·1. $C_{24}H_{39}O_7N_7,3H_2O$ requires C, 49·5; H, 6·0; N, 16·9; OMe, 5·3; H_2O, 9·3%) (Material dried at 100°/0·1 mm. rapidly gained 11·3% in weight. Absorption of $3H_2O$ requires 10·3%).

3 : 4-Diaminoanisole.—3-Nitro-4-aminoanisole (15 g.) was added in small quantities during 0·5 hour to a hot solution of stannous chloride (80 g.) in hydrochloric acid (*d* 1·16; 140 c.c.) which was then heated on the steam-bath for 1 hour. The solution was cooled, basified, and extracted with ether, the extract dried (K_2CO_3), and the solvent removed; the residue distilled at 200—210°/21 mm. The distillate solidified and was crystallised from benzene (some decomp.), yielding reddish crystals (7·6 g.), m. p. 52°. The diamine dihydrochloride (1 g.) and benzil (1 g.) were heated on the steam-bath in aqueous alcohol

(50%; 5 c.c.) for one hour. 6-*Methoxy*-2 : 3-*diphenylquinoxaline* separated from the hot solution; it crystallised from alcohol in yellow prisms, m. p. 156° (Found : N, 8·8. $C_{21}H_{16}ON_2$ requires N, 9·0%).

6-(*or* 7)-*Methoxyalloxazine*.—3 : 4-Diaminoanisole dihydrochloride (6·2 g.) in water (15 c.c.) was added to a solution of alloxan (5·4 g.) in water (15 c.c.). A copious brown precipitate (6·75 g.) rapidly appeared and was filtered off after 6 hours at room temperature. This *product* separated from hot glacial acetic acid (600 c.c.) with solvent of crystallisation which could not be removed in a vacuum at 110°; it was, therefore, recrystallised from pyridine, and formed yellow microcrystals, which fell to a fine powder at 110° in a vacuum and did not melt below 300° (Found : N, 22·6; OMe, 12·8. $C_{11}H_8O_3N_4$ requires N, 23·0; OMe, 12·7%). A similar compound was produced in the condensation of alloxan with (II) in aqueous hydrochloric acid.

The authors thank Mr. S. Bance, B.Sc., A.R.I.C., for the semimicro-analyses, and the Directors of May and Baker Ltd. for permission to publish these results.—THE RESEARCH LABORATORIES, MAY AND BAKER LTD., DAGENHAM, ESSEX. [*Received, November 26th*, 1947.]

OBITUARY NOTICES.

EDWARD CHARLES CYRIL BALY.

1871—1948.

EDWARD CHARLES CYRIL BALY, who died on January 3rd, 1948, had very nearly attained the age of 77, having been born on February 9th, 1871. He was the son of Edward Ely Baly and Alice Mary, daughter of John Winterbottom. At the time of their marriage in 1870, Edward Ely Baly was a clerk in the Bank of England. He was appointed Deputy Chief Cashier in 1879, and the family then came to reside in the official residence at the Bank in Threadneedle Street, since the Chief Cashier refused to occupy it. Here the Baly family lived until the retirement of E. E. Baly in 1895.

In 1883, E. C. C. Baly went to Temple Grove School, East Sheen, and in 1885 obtained a junior Platt Scholarship at Aldenham School, where he won the senior Platt Scholarship in 1886. As he has himself related, he got an excellent training in Latin and Greek under the Head Master of Aldenham, John Kennedy, to whom he owed a deep debt of gratitude. Realising that his interests lay more in science than in classical scholarship, his father took him in 1888 to see Professor Ramsay at University College, London. The result was that he came to University College in 1889 and (at Ramsay's suggestion) studied for the Associateship of the Institute of Chemistry, which he obtained in 1892. Baly's father must have been a man of scientific tastes, for he possessed a 6-inch astronomical telescope with spectroscopic attachment, the result being that his son early gained some knowledge of astronomical spectroscopy. Becoming under Ramsay's influence a skilful glass-blower, he very soon acquired the technique of making and filling " vacuum " tubes with various gases and examining their emission spectra. Although the results were not published till 1893, it seems pretty certain that the young student for the A.I.C. was soon engaged in interesting research work! Baly's ability as an investigator and teacher was recognised by Professor Ramsay. In 1895 he was asked by Ramsay to lecture on inorganic chemistry to junior students, and in 1898 he was appointed Lecturer in theoretical and practical chemistry to medical students. In 1899 he received the title of Assistant Professor, and in 1901 he was appointed a scientific assistant in connection with the practical examinations in chemistry and physics held by the University of London. For his work on the emission spectra of the rare gases, and especially his researches with numerous collaborators on the selective absorption spectra of organic compounds, Baly soon became recognised as an expert on spectroscopy, and established a spectroscopic laboratory at University College. He obtained the title of Lecturer in Spectroscopy in the University of London (1903–10), and gave evening lectures on that subject. His book on Spectroscopy first appeared in 1905 and was highly esteemed. A second edition was published in 1912, and a third edition (in 3 volumes) in 1924–27. His contributions to the advance of science were recognised (in 1909) by election to the Fellowship of the Royal Society. He was also elected a Fellow of University College.

The first time I saw Cyril Baly was in 1895 (or 1896) when I called in at University College on my way to Germany. What I saw was a young man with rather long hair, standing on the top of a step-ladder and pouring sodium (or potassium) amalgam into a glass funnel from which the amalgam dropped down a long glass tube up which streamed a current of ozonised oxygen. I was led to understand that he was trying to prepare metal " ozonides ". So far as I know he never published anything on this subject. It was not till 1899–1900, when I worked in Ramsay's laboratory as a research student, that I first got to know him and appreciate his kindness and his great skill as a glass-blower and constructor of apparatus. My efforts to measure the rates of effusion of argon, helium, and other gases through a minute hole in a platinum disc would have been doomed to failure had it not been for Baly's invaluable help.

In those days (and for many years afterwards) he was a skilled pianist and deeply interested in music, especially the works of Brahms. He owed much to his father, who was a brilliant pianist, and to his mother, who had a beautiful soprano voice. They taught him to play the piano and encouraged him to become proficient. At University College, Professor Arthur Platt and he founded in 1902 the Musical Society, which was a great addition to the amenities of the College, and had a most successful career under their joint care and supervision. There can be no doubt, I think, that an important part of Cyril Baly's temperament was essentially artistic and intuitive.

In 1902 he married Ellen Agnes, only daughter of Thomas Jago, physician and surgeon of

Barnsbury, London. Both father and daughter were musical people. This marriage has been a very happy one. They had four sons, all of whom, and their mother, are living.

In 1910 Cyril Baly was called to Liverpool University, succeeding Campbell Brown as Grant Professor of Inorganic Chemistry. At Liverpool he continued and developed the work on absorption spectra, chemical constitution, and reactivity, including also phosphorescence and fluorescence. Soon after 1920 he took up with great enthusiasm a study of photochemistry (" photochemical catalysis ") and photosynthesis. His last paper on this subject was published in 1939, whilst his whole work in this field was reviewed in his book on " Photosynthesis," published in 1940.

During the first world war, Baly was appointed one of the Deputy Inspectors of High Explosives working under the Chief Inspector, Lt.-Col. C. D. Crozier. His area included Fleetwood, Lytham, Liverpool, N. Wales, and Bristol. For his excellent work in this important department of the production of war munitions he was awarded the C.B.E. in 1918.

At Liverpool University, Baly proved to be a very inspiring and enthusiastic leader in both teaching and research. His lectures were highly appreciated by many generations of students, who were not only kept in touch with the modern advances of chemical science but also strongly attracted by the novelty of many of the lecturer's ideas, the good form and lucidity of his exposition, and the friendly and informal manner of its delivery. Baly knew how to make chemistry a live and exciting subject, one in which new and important discoveries and developments were constantly taking place. He had personally witnessed Ramsay's famous discoveries in the period 1894–1910, and could hold his Liverpool students spell-bound by the story of those great days. Moreover, his students knew that their professor was actively engaged in research, with the result that many of them became his enthusiastic collaborators.

During the twenty-seven years that Baly was Professor in Liverpool many important developments of the Chemistry Department took place. A Professorship in Organic Chemistry was instituted, and in order to accommodate this Department a new and important addition to the old chemistry building was made. The Chair of Organic Chemistry was held successively by R. Robinson, I. M. Heilbron, and A. Robertson (the present holder). There seems little doubt that Baly must have played an important part in these developments.

In 1937 he retired owing to the age limit, and came back to the London area, where he took a house in Wendover, Buckinghamshire. He soon took an active part in the work of the British Association of Chemists, acting as Honorary Registrar for a number of years. During these latter years he also became an examiner in chemistry to the Pharmaceutical Society. Towards the end of the war it became evident to his friends that his health was failing, for he seemed to have considerable difficulty in walking, and the once genial and talkative Cyril Baly became strangely silent.

As mentioned previously, Baly became early interested in the emission spectra of rarefied gases (under electric discharge), and was much intrigued by the fact that oxygen and nitrogen exhibited two spectra, one in the positive column and the other in the negative glow. His first published papers dealt with the separation and striation of rarefied gases under the influence of the electric discharge, and a possible explanation of the twofold spectra of oxygen and nitrogen. He became convinced that these different spectra were caused by " different entities in the chemical sense ". In fact, by using an induction coil giving a 6-inch spark in air, with platinum tubes as electrodes, and passing a slow stream of oxygen at atmospheric pressure into the glass tube and out through the hollow electrodes, he obtained (under certain specified conditions) samples of oxygen of markedly different densities—in one experiment 15·84 from the cathode and 16·12 from the anode (referred to O = 16). This result and others of a similar nature were not published, but were submitted to the Smithsonian Institution in a competition for the Hodgkin Prize. It is curious that such astonishing results were not published by Baly. Very many years afterwards he mentioned them in a public lecture, on which occasion Whytlaw-Gray suggested that they might have been due to a partial separation of isotopes.

During this earlier period at University College Baly published work on the emission spectrum of cyanogen, and on the emission spectra of neon, krypton, and xenon. These spectra of the then newly discovered " rare gases " were measured by means of a ten-foot concave grating, but the results were published before Kayser's criticism of the accuracy of grating measurements was known. Nevertheless, the mounting of this grating and its calibration represented a fine and difficult piece of work. Two other researches of this period may be mentioned : one (with Ramsay) dealing with the composition of the gaseous and liquid phases during the distillation of liquid air, and one (with F. G. Donnan) on the variation with temperature of the surface energies and densities of liquid oxygen, nitrogen, argon, and carbon monoxide.

In 1903 Baly turned his attention to a field of investigation in which the most important scientific work of his life was to be carried out, namely the selective (ultra-violet) absorption spectra of carbon (organic) compounds in the liquid state or in solution, and the relation of these spectra to their chemical constitution and chemical activity. As is well known, the first work in this field was due to Huntington and Hartley (W. N.) in 1879. Hartley devoted his life to it, and his work (and also that of J. J. Dobbie) had amply demonstrated the interest and importance of such measurements. In the session 1901–1902 I had given a few lectures on this subject to advanced students at University College, and suggested to Baly in 1903 that the investigation of such absorption spectra might be more fruitful than that of the emission spectra of gases. This suggestion was eagerly adopted, his first investigation (1904–05), in collaboration with C. H. Desch, dealing with acetylacetone and ethyl acetoacetate, and their metallic derivatives. It was found that the pure " ketonic " form was almost completely diactinic, whilst the pure " enolic " form showed only a small general absorption. When an absorption band was obtained, it was concluded that both forms were present in a state of " dynamical equilibrium ", the band being due to the " change of linking " accompanying the reversible change from one tautomeric modification to the other, and the production of the band being explainable by the conception of the atom as " a system of electrons ". It must be confessed that such a theory seems rather puzzling at first sight, because, if there really existed a rapid *kinetic* interchange between the enol and the keto-forms, as seems to be assumed, one might expect the electronic vibrations to make the solution self-radiating. What Baly and Desch meant, however, was, I think, that the presence of an absorption band was due to the existence of a corresponding *potential* period of (damped) vibration, in the sense of classical resonance. In other words, when an absorption band is formed, the state of affairs in the solution must be such that there exists a possibility of the enol-keto-change of linking, *i.e.*, a potential activity of this type. As a matter of fact, in later years Baly (in collaboration with F. O. Rice) somewhat changed his interpretation of these phenomena in view of the criticisms of Hantzsch and others, and adopted an explanation based on his " force-field " theory (see later).

In a paper published in collaboration with Professor J. Norman Collie (1905), the seven absorption bands of benzene were explained by the assumption that each band is due to a " separate make-and-break of valency links " between the carbon atoms of the ring, caused by " pulsations " of the whole ring. Here again we must assume that the " electronic vibrations " due to these various changes of valency-linking represent potentially existing periods of vibration of the system.

It may be said, I think, that Baly's view as regards the formation of absorption bands (in the ultra-violet or the visible parts of the spectrum) came to a fairly definite form in the papers published (1906) in collaboration with A. W. Stewart. These investigations concerned mainly the absorption bands exhibited in ethyl pyruvate and various organic compounds containing two ketonic groups, *e.g.*, α-diketones and *p*-benzoquinones. The view is expressed that the observed absorption bands originate in " some form of oscillation between the residual affinities of the oxygen atoms of the carbonyl groups ", and for this " oscillation " the name *isorropesis* (meaning " equipoise ") is proposed. This oscillation " results in the establishment of a *free period of vibration* (in the visible region of the spectrum) ". Hence the compounds are coloured. It is to be observed that this more general concept of isorropesis does not involve any actual wandering of an atom, but only a change from one valency distribution to another. In the case of ethyl pyruvate this was expressed by the formulation

In this connection, the authors (Baly and Stewart) say : " It is very hard to indicate what is meant by the aid of the usual structural formulæ, as they only indicate a static condition of the molecule, while what we wish to suggest is essentially a dynamic state. We wish to make it perfectly clear that we do not suppose these two forms necessarily to exist statically; but, owing to the defect of ordinary structural formulæ, it is impossible to write them otherwise if the usual symbols be employed. Our conception can best be comprehended if it be clearly borne in mind that the two formulæ are not intended to represent actual compounds, but merely two phases of the same compound. If this conception of phases be understood, it will be apparent that the change of linkage is continually going on, and that this change will affect the intra-atomic relations of the molecule ".

From these statements we may presume that the continual change of linkage occurs when the molecule is (classically) resonating owing to the absorption of light by the existing " free

period ". I think that is what the authors meant. Referring to this concept of isorropesis, in his Obituary Notice of A. W. Stewart (this vol., p. 396), Professor S. Smiles regards it as " the concept of a state of balance existing in the valency distributions ", and expresses the opinion that it evidently contains a tentative step towards a theory of mesomerism. It is certainly true that Baly was attempting to explain a definite property, *i.e.*, selective absorption of light, by a concept denoting the " equipoise " between different valency distributions, but it must be confessed that his concept of " classical " resonance (very natural in 1906) has little relation to the modern concept of quantum-mechanical " resonance " associated with the name of L. Pauling.

During this period of collaboration with A. W. Stewart, Baly arrived at conclusions having an important bearing on his theory of colour in organic compounds. In order to start the isorropesis, he held that some influence must be present to disturb the residual affinities of the atoms concerned. Given the necessary influence, it followed that all the phenomena of visible colour are due to the oscillation between residual affinities on atoms or groups of atoms in juxtaposition. Moreover, any assumption that two compounds must be fundamentally different in constitution if one is coloured and the other not is quite untrustworthy. As a result, Baly attacked the " quinonoid " theory of colour associated with the name of H. E. Armstrong. Those who were present at the meetings of the Chemical Society in the years 1904–10, when Baly was expounding his theory of isorropesis and extending it to a great variety of organic compounds, will remember the scorn with which Professor Armstrong received the appearance at Burlington House of this strange Greek child from Gower Street.

Although the concept of isorropesis and " isorropic " absorption bands played a notable part in many of these investigations, Baly gradually developed another line of thought which led him to connect absorption spectra with chemical reactivity. This new theory originated in an investigation, in collaboration with Miss E. G. Marsden, on certain amino-aldehydes, and in work on the sulphonation of phenols in collaboration with F. O. Rice. In the former case it was found that if a small quantity of an alcoholic solution of hydrogen chloride be added to the alcoholic solution of any one of the amino-bases investigated, a new absorption band appears, but on addition of excess of acid the spectrum changes to that of the hydrochloride of the amino-base. According to Baly, the first small portion of acid produces a definite intermediate phase in the " opening-up " of the (" electromagnetic ") field of force of the amino-aldehyde molecule, the alcohol of the solution having already opened it up to a first degree or phase. This higher phase produced by the small quantity of acid then reacts with the excess of acid to form the definite salt (hydrochloride). The analogous phenomena observed in the sulphonation experiments were explained in a similar manner. From these results Baly drew the general conclusion that " in all chemical reactions the force-fields of the reactant molecules have to be opened up to definite stages before any reaction can take place, and that the changes in the absorption of light observed are due to the existence of these stages and not to any change in the primary structure of the molecule ".

This " force-field theory " led Baly to an interesting general theory of chemical reactivity. A molecule is unreactive if its field of force is " closed ". The opening-up of the field, and hence the reactivity of the molecule, can be effected (in definite stages) by the action of a solvent, by various chemical reagents, and by absorption of light. This activation of molecules by the opening-up of their force-fields might evidently afford an explanation of catalytic actions. Moreover, a " de-activation " of molecules, by the closing-up of their force-fields owing to the interlocking of the latter with the force-fields of new substitutent groups, might also occur. It was obvious to Baly that all such actions could be closely related to the presence or absence of absorption bands, and this led to a further vigorous development of his work in this field.

An interesting example of the application by Baly of the force-field theory is afforded by his criticism of some of Hantzsch's results. Hantzsch had obtained a number of differently coloured salts of diphenylvioluric acid, and had explained these differences by *different* structural formulæ. The following quotation will explain Baly's view. " The stages in the opening-up of the closed force-field of a complex acidic substance will depend on the strength or affinity of the base employed. Thus, the hydroxides of lithium, sodium, potassium, rubidium, and cæsium are in ascending order of magnitude as far as their affinity as bases is concerned. It follows that the effect of these may be different as regards the opening-up of a complex acidic force-field. This at once affords an explanation of Hantzsch's differently coloured diphenylviolurates, for the more electropositive the metal the higher the stage that will be called in to play, and hence the longer the wave-length of the light that will be selectively absorbed. There is no need for any

difference in the structural formulæ of the salts, for they only differ in the condition of the force-field of the complex acid."

Another example of Baly's conception of the various phases of the opening-up of a molecular field of force, both by the action of solvent molecules and by light, is afforded by his paper, published in collaboration with R. Krulla (1912), and entitled " A theory of fluorescence ". The reasoning here is very tentative, but the views expressed are interesting since they emphasise the concept that when light opens up the field from one phase to another it expends free energy, *i.e.*, does work on the system, so that the higher phase of the field is richer in energy. This paper was followed by a long series of papers dealing with light-absorption and fluorescence, culminating in an elaborate paper entitled " A theory of absorption, fluorescence, and phosphorescence " (1915). In these papers (and particularly in the last one) Baly made use of the force-field theory and also of ideas derived from the theory of energy quanta. I think it is correct to say that he was led into quantum considerations by his supposition, based on quite inadequate evidence, that the " central " frequencies of his observed absorption bands in the ultra-violet are integral multiples of a " basic " frequency in the infra-red. In his important work of that period, Bjerrum had put forward the theory that the *rotational* frequencies of the long infra-red bands are integral multiples of a basic value, but Baly erred in assuming that the frequencies of the *atomic and electronic oscillations* (bands) are integral multiples of the same basic value. This extension of Bjerrum's theory was quite unjustified. It is probably correct to say that Baly's work of 1912–15 was an interesting, and, for its time, valuable contribution, but that it contained good and bad ideas mixed together in a confusing way, and an erroneous extension of Bjerrum's theory of 1912. One must remember, however, that these were very early days in the development of quantum theory, and that Baly was not a theoretical physicist. He made a gallant and in many ways pioneer attempt to solve a difficult and complicated problem.

In this brief sketch of the very great amount of original work carried out on absorption spectra in liquid systems by Baly and his numerous students and collaborators, I have not attempted to go into details of experimental methods or results. He made valuable improvements in the methods used by his predecessors, and his results are in general trustworthy so far as they go. Owing to the nature of the systems investigated and the technique mainly employed, the " absorption bands " as he observed them could only indicate rather general regions of selective absorption. It would not have been possible therefore to apply any exact *quantitative* theory to results of this type, even if such a theory had existed then. But Baly's theories of isorropesis and molecular fields of force, although they were necessarily of a rather qualitative and " elastic " character, enabled him to make many interesting deductions relating to chemical constitution, processes, and reactivity. Although he sometimes made mistakes of interpretation (and some of actual fact), he was an energetic and imaginative pioneer in the search for the hidden " dynamical " causes underlying the observed absorptions, rather than a seeker for mere analogies of a purely " statical " character between the observed spectra and " statical " structural formulæ. I think the future historians of chemistry will assign a very definite value to his prolonged and strenuous effort, even though the modern electronic and quantum-mechanical theories of chemical science have solved the type of problems he investigated in a different manner.

In 1920 Baly became deeply interested in the problem of the photosynthetic production of carbohydrates and other organic compounds (proteins and alkaloids) in the green leaves of living plants exposed to light. Many years previously, von Baeyer had suggested that the first stage is the formation of formaldehyde, which is then polymerised to hexoses, and this theory had been partly confirmed by the work of Willstätter and Stoll. In 1913 Benjamin Moore (in collaboration with Webster) had found that if an aqueous solution of carbon dioxide be exposed to direct sunlight for some days no formation of formaldehyde can be detected, but if colloidal ferric hydroxide or uranium hydroxide be present small quantities of formaldehyde are produced. It was, I think, this work of Moore and Webster which chiefly aroused Baly's interest, since such a photochemical reaction seemed difficult to explain. Hence, in collaboration with I. M. Heilbron and W. F. Barker, he tried the effect of short-wave ultra-violet light, because it was known that carbon dioxide absorbs light in the region of 2100 A. It was found that when a stream of carbon dioxide was passed through water contained in quartz tubes and irradiated by the light from a quartz mercury lamp, small quantities of formaldehyde could be detected in the solution. New results were obtained when certain " white " powders, such as aluminium hydroxide and zinc and magnesium carbonates, were kept suspended in the solution during the irradiation. When the resulting solutions were filtered and evaporated, 0·02 g. of a " gummy residue " was obtained, which on being heated with dilute hydrochloric acid gave " reactions

for carbohydrates ". Baly attributed the success of these experiments to the adsorption of the carbonic acid on the surface of the powders, and concluded that this adsorption was necessary for the formation of carbohydrates by the action of light.

Meanwhile C. W. Porter and H. C. Ramsperger, in the University of California, had repeated the earlier experiments on the action of short ultra-violet light on an aqueous solution of carbon dioxide, and had found that, when stringent precautions are taken to avoid the entrance of any trace of impurity, no formaldehyde is produced. Hence they concluded that in the Liverpool experiments the formation of formaldehyde must have been due to the presence of some unknown impurity. To this criticism Baly had several answers. One was as follows: " There is no doubt that in this reaction the carbonic acid is converted directly into carbohydrates and that formaldehyde as such is not produced, and it seemed that the most probable explanation of the discrepancy between our results and those of Porter and Ramsperger was that the action of the ultra-violet light is to establish a photochemical equilibrium, $6H_2CO_3 \rightleftharpoons C_6H_{12}O_6 + 6O_2$, which reverts to carbonic acid again in the dark. In the presence of oxidisable impurities a small amount of carbohydrates will be formed, which will be photochemically decomposed to formaldehyde. This decomposition of all the carbohydrates by means of ultra-violet light is well known ". Another explanation of the discrepancy was: " The ' impurities ' mentioned by Porter and Ramsperger were not impurities in the ordinary sense, but all the suspended dust particles had been removed from the solution. This was not done in the experiments carried out by Heilbron, Barker, and myself, with the result that sufficient surface was present to give a minute amount of photosynthesized products ". It must be confessed that such explanations do not appear very satisfactory.

The research took a new turn when, as a result of a suggestion made by Dr. W. E. Stephen (one of Baly's collaborators), a *green* powder (nickel carbonate) was used instead of the previously mentioned white powders, and the light from an ordinary tungsten-filament lamp replaced that from the quartz mercury lamp. Glass vessels could now be used. It was the colour of chlorophyll that suggested the use of a green substance, but it was found that pink cobalt carbonate gave equally good results. The yield of " carbohydrate material " now obtained was better than that given by the white powders in ultra-violet light, the best yield being 0·075 g. in two hours. From the products a solid osazone was obtained. A particularly good form of nickel carbonate was prepared by an electrolytic method, since Baly had found that small amounts of alkalis (alkali carbonate) had a deleterious effect.

Being greatly impressed by these results, I asked Baly's permission to allow us at University College, London, to develop them further by employing different parts of the visible spectrum. This permission having been obtained, the research was undertaken by Dr. James Bell, who naturally began by repeating Baly's work, employing all his methods and precautions, and using besides his kieselguhr-supported catalysts the best form of nickel carbonate. The startling result was that Bell could find no trace of the formation of any organic matter whatsoever. An even more startling result was that when Baly heard of these negative results he tried to repeat his former work with the electrolytically prepared nickel carbonate and he also obtained negative results. Nor was he able to give any satisfactory explanation of the discrepancy.

As a result of this state of affairs, Baly now directed his attention almost wholly to the investigation of certain oxides supported on kieselguhr, and finally claimed to be able to prepare a photosynthetically active material by coating kieselguhr with an anhydrous layer of NiO containing ThO_2 in the molecular ratio of $1ThO_2$: 24NiO, and that " when kieselguhr, which is completely coated with a trimolecular layer of these oxides, is irradiated in presence of water saturated with carbon dioxide, by daylight or the light of a tungsten filament lamp, there is photosynthesised a carbohydrate ".

In concluding this account of Baly's researches on " artificial " photosynthesis, reference may be made to his earlier papers on " The photosynthesis of nitrogen compounds from nitrates and carbon dioxide ", and " The photosynthesis of naturally occurring nitrogen compounds from carbon dioxide and ammonia ". Mention should also be made of his experiments on the action of ultra-violet light on a 40% solution of formaldehyde. The complex product of this action was investigated in the laboratory of Sir James Irvine at St. Andrews University, and was found to contain some hexoses, amongst much other material. As the solution during irradiation was prevented from becoming acid by the presence of magnesium or calcium carbonate, the possibility that the action was chiefly an exothermic auto-condensation in a slightly warmed alkaline solution cannot, perhaps, be excluded.

In this brief account of Baly's researches on photosynthesis I have tried to write as objectively as possible, as my personal contact with it was confined to the work of Bell; but I am

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